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(71) Applicant: **ONO PHARMACEUTICAL CO., LTD.**
1-5, Doshomachi 2-chome
Chuo-ku Osaka 541 (JP)

(72) Inventor: **Kawamura, Masanori Ono Pharmaceutical Co. Ltd.**
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka (JP)

Arai, Yoshinobu Ono Pharmaceutical Co. Ltd.
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka (JP)

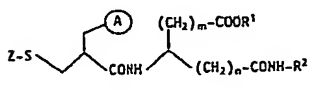
Aishita, Hideki Ono Pharmaceutical Co. Ltd.
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka (JP)

(74) Representative: **Bentham, Stephen et al**
J.A. Kemp & Co. 14 South Square Gray's Inn
London WC1R 5EU (GB)

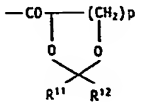
Claims for the following Contracting States: ES + GR.

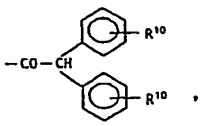
(54) Novel amino acid derivatives.

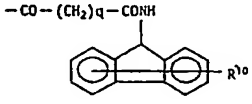
(57) Compounds of the formula:

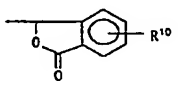


(wherein R^1 is hydrogen or alkyl; R^2 is an optionally substituted carbocyclic or heterocyclic ring; Z is hydrogen or a group $-COR^9$ (R^9 is alkyl or phenyl)









in which R^{10} is hydrogen, halogen, trihalomethyl, alkyl or alkoxy, R^{11} and R^{12} are each hydrogen, alkyl or phenyl substituted by R^{10}, or R^{11} and R^{12} together represent alkylene and p is 1 or 2; q is 1 to 4; A is phenyl or cycloalkyl optionally substituted by halogen, trihalomethyl, alkyl or alkoxy; and m is zero and n is 1 to 4 or n is zero and m is 1 to 4; and non-toxic salts thereof, have an inhibitory effect on enkephalinase and are useful as analgesic, antianxiety and anticonvulsant agents.

EP 0 341 081 A2

Description

NOVEL AMINO ACID DERIVATIVES

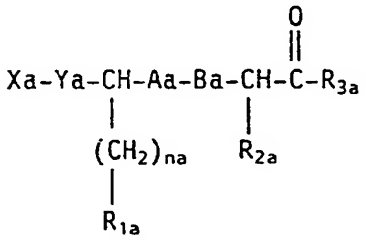
This invention relates to novel amino acid derivatives having an inhibitory activity on enkephalinase, to processes for their preparation, pharmaceutical compositions containing them and to their use.

"Enkephalin" is a general term referring to compounds of the formula:
Try-Gly-Gly-Phe-A
(wherein A represents Met (in Met^5-enkephalin) or Leu (in Leu^5-enkephalin)). These two pentapeptides bind to opioid receptors and thereby produce an analgic effect. They are neurotransmitters (see Nature, 258, 577 (1975)).

Enkephalinase, discovered by Malfroy et al, is an enzyme which cleaves Met-enkephalin or Leu-enkephalin at the Gly^3-Phe^4 bond (see Nature, 276, 523 (1978)). It plays an important role in the termination of the analgesic effect which enkephalin has. It is considered that the inhibition of enkephalinase slows down the deactivation of enkephalin and that the analgesic effect is maintained.

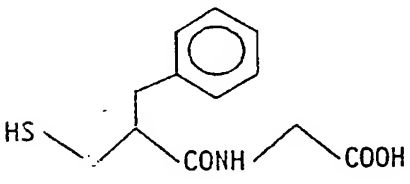
On this basis, there has recently been active research and development on enkephalinase inhibitors.

For example, in European Patent Publication No. 38758, the compounds of the general formula:



(a)

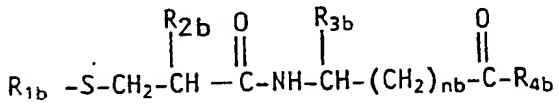
[wherein
Xa-Ya represents, inter alia, a mercapto group,
na represents zero or one,
Aa-Ba represents, inter alia, a CONH group,
R_{1a} represents, inter alia, a hydrogen atom, or optionally substituted alkyl group or an optionally substituted phenyl group,
R_{2a} represents a hydrogen atom, an alkyl group, a phenyl group, an optionally substituted benzyl group, a hydroxyalkyl group, an optionally substituted alkoxyalkyl group, a phenoxyalkyl group or an optionally substituted mercaptoalkyl group,
R_{3a} represents a group of the formula: OR_{4a}, NHR_{4a} or $N(R_{4a})_2$ in which R_{4a} represents, inter alia, an optionally substituted phenyl)are proposed. In particular thiorphan having the formula:



is noted (see Nature, 288, 286 (1980)).

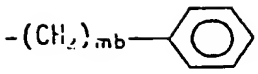
Thereafter, compounds wherein the glycine moiety in thiorphan is replaced by various substituents, have been proposed.

For example, in European Patent Publication No. 136883 (and Japanese Patent Kokai No. 60-136554), the compounds of the general formula:

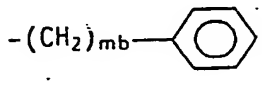


(b)

(wherein R_{1b} represents a hydrogen atom or
R_{5b}-C(=O)-.
R_{2b} represents, inter alia, a group of the formula:



R_{3b} represents a hydrogen atom, an alkyl group, an alkyl group of the formula: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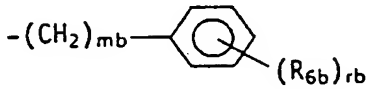
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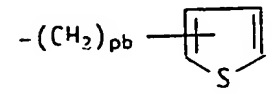
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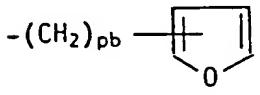
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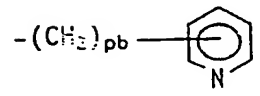
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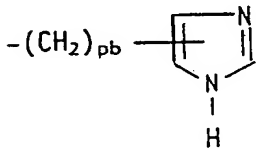
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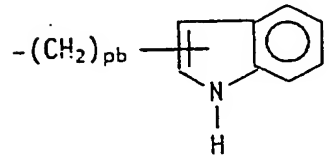
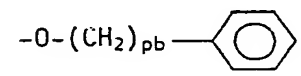
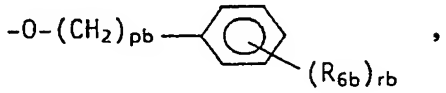


or $-(CH_2)_{pb}$-cycloalkyl,
R_{4b} represents a hydroxy group, an alkoxy group a group of the formula: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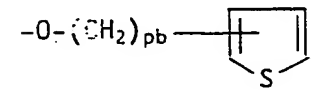
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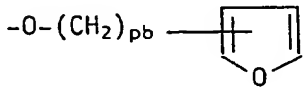
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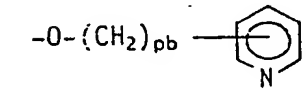
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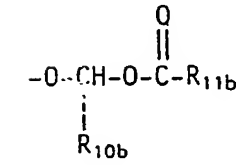
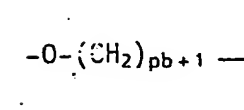

,
$-O-(CH_2)_{pb+1}-OH$,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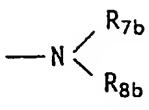
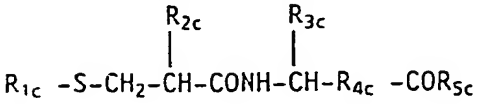


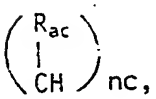
and nb represents an integer of 1 to 15) are proposed.

In South African Patent Publication No. 840670, the compounds of the general formula:

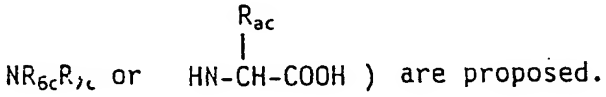


(c)

(wherein
R_{1c} represents a hydrogen atom, an acyl group or an aroyl group,
R_{2c} represents a hydrogen atom, an alkyl group, an alkenyl group, an alkynyl group or aralkyl group,
R_{3c} represents a hydrogen atom, an alkyl group, a carboxy group, a carboxyamido group, a substituted alkyl group, a substituted aryl group, a thiol group, an alkylthio group or a heteroaryl group,
R_{4c} represents a group of the formula:

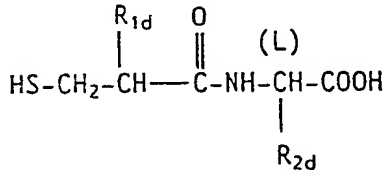


nc represents an integer of 1 to 3, and
R_{5c} represents a hydroxy group, an alkoxy group, an aryloxy group, an aralkyloxy group, an NH_2 group, or a group of the formula:



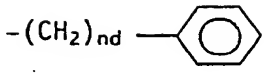
are proposed.

In the U.S. Patent No. 4401677, the compound of the general formula:



(d)

(wherein
R_{1d} represents an alkyl group, a benzyl group or a phenethyl group,
R_{2d} represents an alkyl group or a group of the formula: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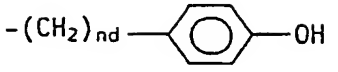
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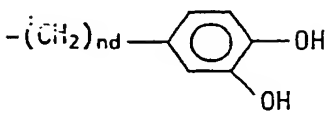
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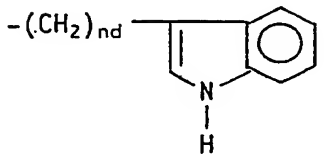
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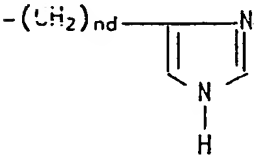
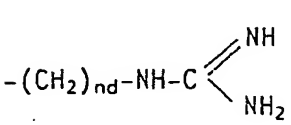
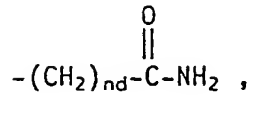

,

$-(CH_2)_{nd}-NH_2$, $-(CH_2)_{nd}-SH$, $-(CH_2)_{nd}-S-$ alkyl,


or

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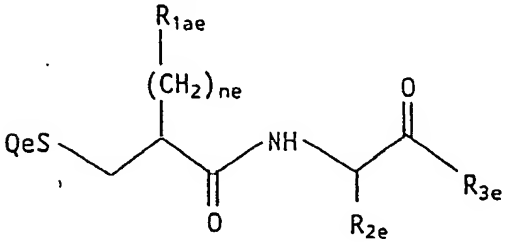
and nd represents an integer of 1 to 4) are proposed.

Further, in European Patent Publication No. 254032, the compounds of the general formula:


(e)

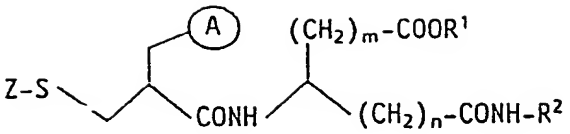
(wherein
R_{1ae} represents a phenyl group substituted by, inter alia, alkyl, alkoxy or cycloalkyl.
R_{2e} represents, inter alia, a group of the formula:
$R_{13e}CONH(CH_2)_{qe}$-, $R_{13e}NHCO(CH_2)_{qe}$-, $R_{6e}OCO(CH_2)_{qe}$-
R_{3e} represents, inter alia, a group of the formula:
OR_{7e}, $-NR_{7e}R_{8e}$,
R_{13e} represents, inter alia, a group of the formula:
Y_{1e}-C_3H_4-,
R_{6e}, R_{7e} and R_{8e} represent, independently, inter alia, a hydrogen atom, an alkyl group or an arylalkyl group,
ne represents zero or an integer 1 or 2,
qe represents an integer 1 to 4,
Qe represents a hydrogen atom or a group of the formula:
$R_{10e}CO$-,
R_{10e} represents, inter alia, an alkyl group or a group of the formula: Y_{3e}-C_6H_4-,
Y_{1e} and Y_{3e} represent independently, inter alia, a hydrogen atom, an alkyl group, cycloalkyl group or an alkoxy group) are proposed.

As a result of research and experimentation it has been found that derivatives wherein

(1) a glycine moiety in thiorphan is replaced by an acidic α-amino acid (e.g. aspartic acid, glutamic acid etc.) and further

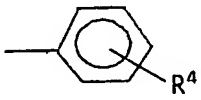
(2) either carboxyl group of the said acidic amino acids is converted into an amido bond with various aromatic amines, have an inhibitory effect on enkephalinase.

Accordingly, the present invention relates to the amino acid derivatives of the general formula:

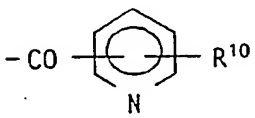


(I)

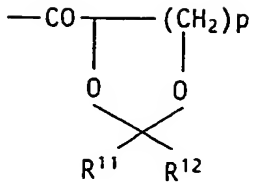
(wherein
R^1 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
R^2 represents a carbocyclic or heterocyclic ring, unsubstituted or substituted by 1 to 3 substituents R^3.
R^3 represents independently;
(1) a halogen atom,
(2) a trihalomethyl group,
(3) a hydroxy group,
(4) an alkyl group of 1 to 15 carbon atoms,
(5) an alkoxy group of 1 to 4 carbon atoms,
(6) an alkylthio, alkylsulfinyl or alkylsulfonyl group, of 1 to 4 carbon atoms,
(7) a group of the formula:



in which R^4 represents a hydrogen atom, a halogen atom, a trihalomethyl group, a hydroxy group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms
(8) a group of the formula:
$-NR^5R^6$
in which R^5 and R^6 independently represent a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(9) a group of the formula:
$-CO-R^7$
in which R^7 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^4 (in which R^4 is as hereinbefore defined),
(10) a group of the formula:
$-COOR^8$
in which R^8 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(11) a group of the formula:
$-CONR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(12) a group of the formula:
$-SO_2NR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(13) a cyano group,
(14) a nitro group, or
(15) a group of the formula:
$-NHCO-R^7$,
in which R^7 is as hereinbefore defined, Z represents:
(1) a hydrogen atom,
(2) a group of the formula:
$-COR^9$
in which R^9 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms,
(3) a group of the formula:

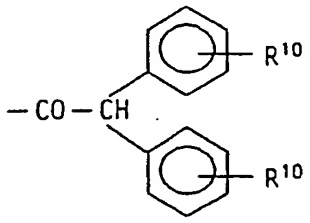


in which R^{10} is as hereinbefore defined,
(4) a group of the formula:



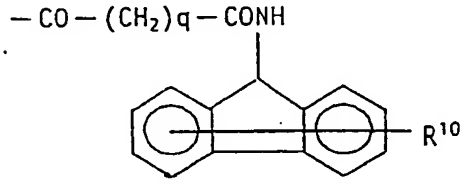
in which R^{11} and R^{12} independently represent a hydrogen atom, an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} is as hereinbefore defined, or R^{11} and R^{12} together represent an alkylene group of 4 or 5 carbon atoms and p is an integer of 1 or 2,

(5) a group of the formula:



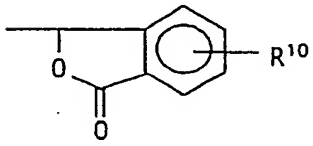
in which the two R^{10} groups are independently as hereinbefore defined,

(6) a group of the formula:



in which R^{10} is as hereinbefore defined and q is an integer of 1 to 4,

(7) a group of the formula:



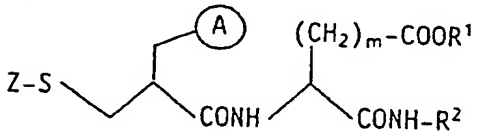
in which R^{10} is as hereinbefore defined,

A represents a phenyl group or cycloalkyl group of 4 to 7 carbon atoms, each of which is substituted by R^{13}, in which R^{13} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms, and

(1) when m is zero, n is an integer of 1 to 4, and

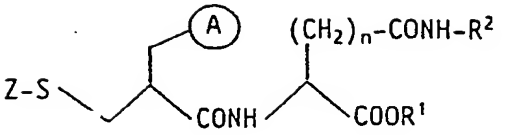
(2) when n is zero, m is an integer of 1 to 4), or a non-toxic salt thereof.

The derivatives of the general formula (I) can be classified into two groups, i.e., α-amide derivatives of the general formula:



(Ia)

(wherein the various symbols are as hereinbefore defined) and derivatives having an amido bond introduced into the β-position or after, of the general formula:



(Ib)

(wherein the various symbols are as hereinbefore defined).

There is no description of compounds of the general formula (Ia) in any of the prior art disclosing the general formulae (a) and (e) mentioned above.

A part of the compounds of general formula (Ib) are broadly embraced by the disclosure in European Patent Publication No. 254032 (the general formula (e) hereinbefore described). However, the description in the European Patent Publication is very broad and no compounds having an amido bond introduced into the β-position or after are in fact prepared; nor is there any disclosure of the biological activities of such compounds. We have synthesized many compounds in the general formula (Ib) and have demonstrated in screening tests that they have a useful biological activity.

In the general formula (I), the alkyl groups of 1 to 4 carbon atoms, represented by R^1, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12} and R^{13} are methyl, ethyl, propyl and butyl groups, and isomers thereof.

The alkoxy groups of 1 to 4 carbon atoms, represented by R^3, R^4, R^{10} and R^{13} are methoxy, ethoxy, propoxy and butoxy groups, and isomers thereof.

The alkylthio groups of 1 to 4 carbon atoms, represented by R^3 are methylthio, ethylthio, propylthio and butylthio groups, and isomers thereof; the alkylsulfinyl groups of 1 to 4 carbon atoms are methylsulfinyl, ethylsulfinyl, propylsulfinyl and butylsulfinyl groups, and isomers thereof; and the alkylsulfonyl groups of 1 to 4 carbon atoms are methylsulfonyl, ethylsulfonyl, propylsulfonyl and butylsulfonyl groups, and isomers thereof.

The alkyl groups of 1 to 15 carbon atoms, represented by R^3 are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl and pentadecyl groups, and isomers thereof.

The halogen atoms represented by R^3, R^4, R^{10} and R^{13} are fluorine, chlorine, bromine and iodine atoms, and the trihalomethyl groups represented by R^3, R^4, R^{10} and R^{13} are trifluoromethyl, trichloromethyl, tribromomethyl and triiodomethyl groups.

The alkylen group of 4 or 5 carbon atoms, represented by R^{11} and R^{12} together, are tetramethylene or pentamethylene group.

In the general formula (I), the carbocylic rings represented by R^2 are generally mono-, bi- or tri-cyclic aromatic carbocyclic rings containing not more than 15 carbon atoms, which may be partially or fully saturated.

Examples of the rings mentioned above are benzene, naphthalene, indene, azulene, fluorene, phenanthrene, anthracene, acenaphthalene, biphenylene rings and partially or fully saturated rings thereof.

More preferably, the carbocyclic ring is a mono-, bi or tri-cyclic aromatic ring composed of benzene skeletons, i.e. benzene, naphthalene, phenanthrene and anthracene.

In the general formula (I), the heterocyclic rings represented by R^2 are generally mono-, bi- or tri-aromatic heterocyclic rings containing not more than 15 carbon and hetero atoms; the rings may be partially or fully saturated.

Examples of the rings mentioned above are furan, thiophene, pyrrole, oxazole, isoxazole, thiazole, isothiazole, imidazole, pyrazole, furazan, pyran, pyridine, pyridazine, pyrimidine, pyrazine, indole, isoindole, benzofuran, benzothiophene, indolizine, benzimidazole, benzthiazole, benzoxazole, chromene, quinoline, isoquinoline, quinolizine, purine, indazole, quinazoline, cinnoline, quinoxaline, phthalazine, pteridine, benzodiazepine carbazole, acridine, phenanthridine, xanthene, phenazine and phenothiazine rings and partially or fully saturated rings thereof.

More preferably, the hetorocyclic ring is mono- cyclic or bi-cyclic incorporating a benzene ring, and contains one or two nitrogen and/or sulfur atoms.

Especially preferred rings represented by R^2 are benzene, naphthalene, furan, thiophene, pyridine, pyrimidine, pyrazine, benzimidazole, benzthiazole, benzoxazole and benzodiazepine rings and partially saturated rings thereof.

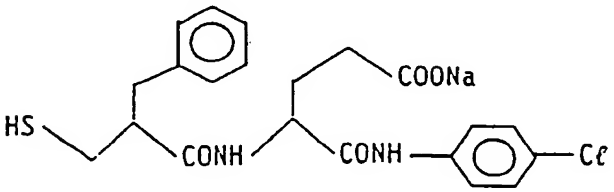
In the general formula (I), the cycloalkyl groups of 4 to 7 carbon atoms, represented by A are cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl groups.

When n is zero, A preferably represents a phenyl group or a cycloalkyl group of 4 to 7 carbon atoms, each of which is substituted by R^{13} (R^{13} being as hereinbefore defined) and when m is zero, A preferably represents an unsubstituted phenyl group or a cycloalkyl group of 4 to 7 carbon atoms which is substituted by R^{13} (R^{13} being as hereinbefore defined).

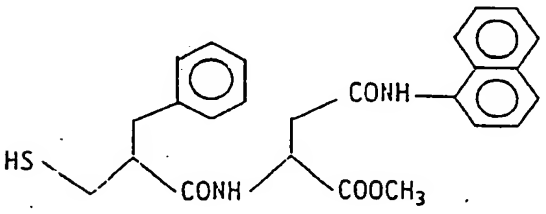
The compounds of general formula (I) may be converted into the corresponding salts by known methods. Non-toxic and water-soluble salts are preferable. Suitable salts include alkali metal salts (e.g. sodium or potassium), alkaline earth metal salts (e.g. calcium or magnesium), ammonium salts, salts of pharmaceutically acceptable organic amines (e.g. tetramethylammonium, triethylamine, methylamine, dimethylamine, cyclopentylamine, benzylamine, phenethylamine, piperidine, monoethanolamine, diethanolamine, tris(hydroxymethyl)aminomethane, lysine, arginine or N-methyl-D-glucamine). Non-toxic acid addition salts are also suitable when the compounds of general formula (I) comprise a basic group, for example when Z represents a nicotinoyl group. Suitable salts include the acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulphuric acid, phosphonic acid and nitric acid, and the salts with

organic acids such as acetic acid, propionic acid, lactic acid, tartaric acid, citric acid, benzoic acid, methansulphonic acid, ethanesulphonic acid, benzenesulphonic acid, toluenesulphonic acid, isethionic acid, glucuronic acid and gluconic acid.

The compounds of general formula (I) can be named as derivatives of an amino acid. For example, the compound of the formula:



can be called N-(3-mercapto-2-benzylpropionyl)-α-(4-chloroanilino) glutamic acid γ-sodium salt, and the compound of the formula:

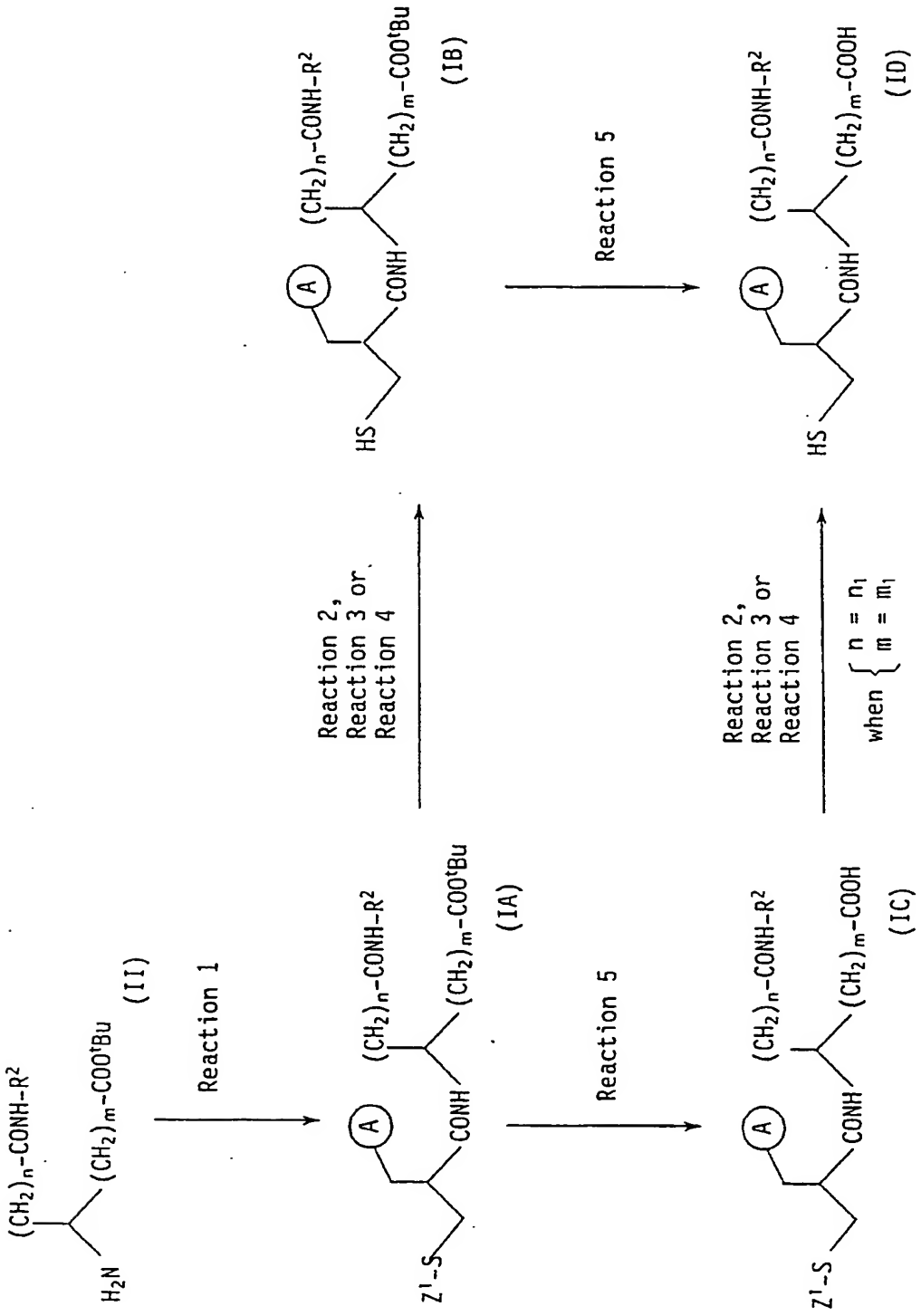


can be called N-(3-mercapto-2-benzylpropionyl)aspartic acid β-(1-naphthyl)amide α-methyl ester. Compounds of the invention in which the amino acid has the L-configuration are preferred.

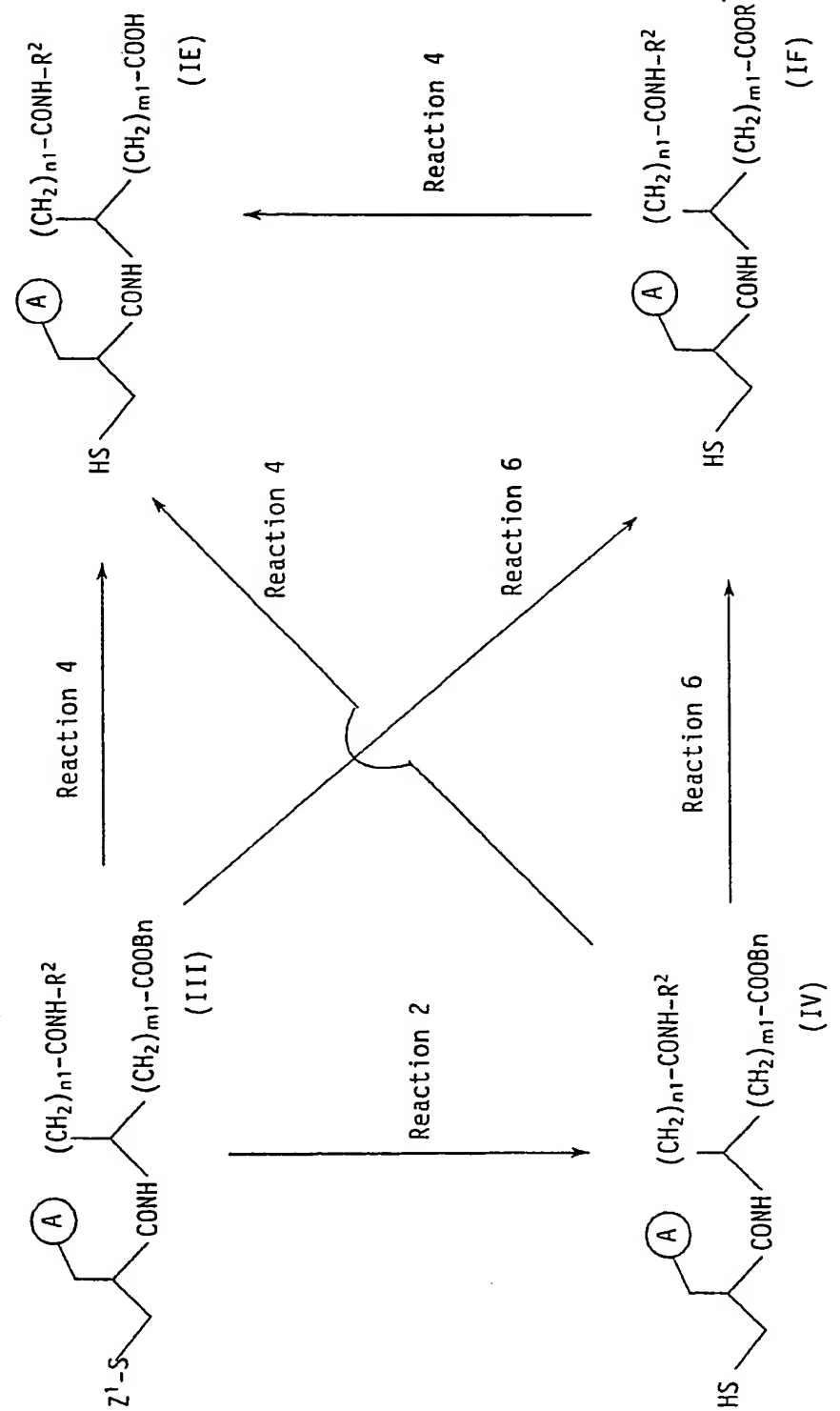
Throughout the specification including the claims, it is to be understood that the alkyl, alkoxy, alkylthio, alkylsulfinyl and alkylsulfonyl groups may be straight- or branched-chain. It is also to be understood that the invention includes all isomers (and mixtures thereof) arising from, for example, the presence of asymmetric carbons in general formula (I).

According to a feature of the present invention, the compounds of the general formula (I) may be prepared by using a series of reactions depicted in Scheme A, B and C below, wherein $R^{1'}$ represents an alkyl group of 1 to 4 carbon atoms,
R^{14} represents a silyl group substituted by three substituents which are selected from an alkyl group of 1 to 4 carbon atoms and a phenyl group (e.g. a tert-butyldimethylsilyl or diphenyl-tert-butylsilyl group),
Z^1 represents a group of the formula: $-COR^9$ (in which R^9 is as hereinbefore defined),
Z^2 represents a group within the definition of Z as hereinbefore defined with the exception of hydrogen,
^{t}Bu represents a tert-butyl group,
Bn represents a benzyl group, and
(1) when m_1 is zero, n_1 is an integer of 1 to 4, and
(2) when n_1 is zero, m_1 is an integer of 2 to 4, and the other symbols are as hereinbefore defined.

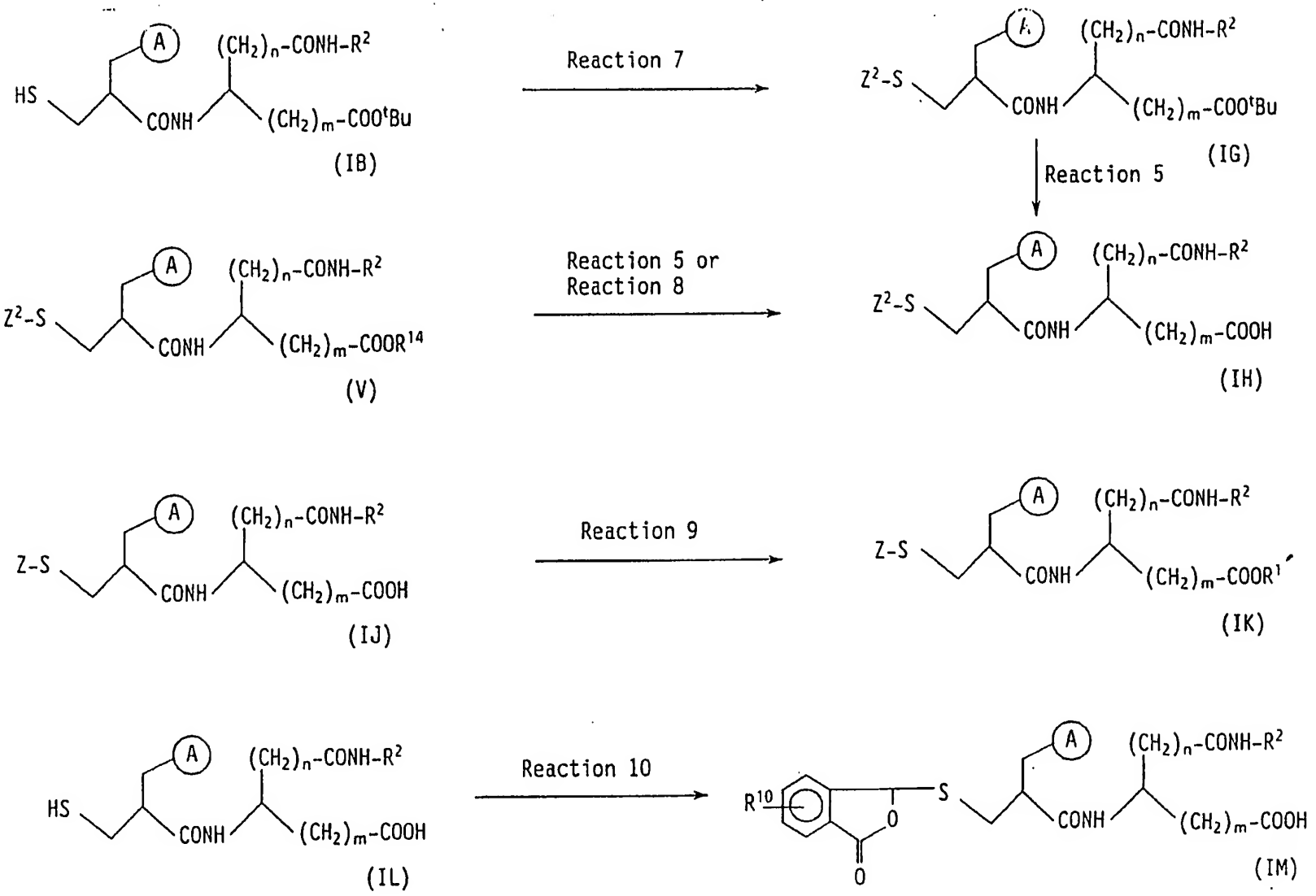
Scheme A


(CH2)n-CONH-R2
(CH2)m-COOtBu
H2N
(II)
Reaction 1
(CH2)n-CONH-R2
(CH2)m-COOtBu
A
CONH
Z1-S
(IA)
Reaction 2,
Reaction 3 or
Reaction 4
(CH2)n-CONH-R2
(CH2)m-COOtBu
A
CONH
HS
(IB)
Reaction 5
(CH2)n-CONH-R2
(CH2)m-COOH
A
CONH
Z1-S
(IC)
Reaction 2,
Reaction 3 or
Reaction 4
when n = n1
m = m1
(CH2)n-CONH-R2
(CH2)m-COOH
A
CONH
HS
(ID)

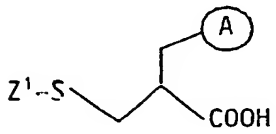
Scheme B


Reaction 4
Reaction 4
Reaction 6
Reaction 2
Reaction 6
Reaction 4
(IE)
(III)
(IV)
(IF)
A
CONH
HS
Z'-S
$(CH_2)_{n1}$-CONH-R^2
$(CH_2)_{m1}$-COOH
$(CH_2)_{m1}$-COOBn
$(CH_2)_{m1}$-COOR'

Scheme C


Reaction 7
Reaction 5
Reaction 5 or
Reaction 8
Reaction 9
Reaction 10
(IB)
(IG)
(V)
(IH)
(IJ)
(IK)
(IL)
(IM)

Each of the steps depicted in the said schemes, are well known to those skilled in the art. For example, Reaction 1 may be carried out by reacting an amine of the formula (II) with a carboxylic acid of the formula:



(VI)

(wherein the various symbols are as hereinbefore defined) to form an amido bond. The said reaction is known and can be carried out by various methods for example:

(A) using a mixed acid anhydride,

(B) using an acid halide,

(C) using a condensing agent such as dicyclohexylcarbodiimide (DCC).

Each of these methods can be carried out, for example, as follows:

(A) the method using a mixed acid anhydride may be carried out, for example, by reacting a carboxylic acid of the general formula (VI) and an acid halide (e.g. pivaloyl chloride, tosyl chloride or mesyl chloride) or an acid derivative (e.g. ethyl chloroformate or isobutyl chloroformate) in the presence of a tertiary amine (e.g. pyridine, triethylamine or picoline) in an inert organic solvent (e.g. chloroform, methylene chloride, diethyl ether or THF) or without a solvent at a temperature of from 0°C to 40°C, and then by reacting the mixed acid anhydride obtained with an amine of the general formula (II) in an inert organic solvent (e.g. as hereinbefore described), at a temperature of from 0°C to 40°C,

(B) the method using an acid halide may be carried out, for example, by reacting a carboxylic acid of the general formula (VI) with an acid halide (e.g. thionyl chloride or oxalyl chloride) in an inert organic solvent (as hereinbefore described) or without a solvent at from -20°C the reflux temperature of the solvent, and then by reacting the acid halide obtained with an amine of the general formula (II) in the presence or absence of a tertiary amine (as hereinbefore described) in an inert organic solvent (as hereinbefore described), at a temperature of from 0°C to 40°C and

(C) the method using a condensing agent such as DCC may be carried out, for example, by reacting a carboxylic acid of the general formula (VI) with an amine of the general formula (II) using DCC in the presence or absence of a tertiary amine (as hereinbefore described), in an inert organic solvent (as hereinbefore described) or without a solvent, at a temperature of from 0°C to 40°C.

The reaction (A), (B) and (C) hereinbefore described are preferably carried out in an atmosphere of inert gas (e.g. argon or nitrogen) under anhydrous conditions.

Reaction 2 may be carried out by reaction of the compound of formula IA with 2-mercaptoethylamine ($HS-(CH_2)_2-NH_2$) in an inert organic solvent (e.g. methylene chloride or acetonitrile), at a temperature of from ambient to 60°C.

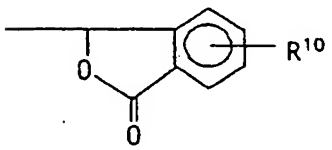
Reaction 3 may be carried out by reaction with anhydrous potassium carbonate or anhydrous sodium carbonate in an absolute alkanol (e.g. absolute methanol or absolute ethanol), generally at a temperature of from -10°C to 100°C.

Reaction 4 may be carried out by using an aqueous solution of an alkali (e.g. potassium hydroxide, sodium hydroxide, lithium hydroxide, potassium carbonate or sodium carbonate) in a water-miscible organic solvent (e.g. dimethoxyethane, THF (tetrahydrofuran), dioxan or a lower alkanol), generally at a temperature of from -10° to 100°C.

Reaction 5 is a hydrolysis under acidic conditions, and may be carried out by reaction with an aqueous solution of an organic acid (e.g. acetic acid, oxalic acid, p-toluenesulfonic acid or trifluoroacetic acid), or with an aqueous solution of an inorganic acid (e.g. hydrochloric acid or, sulfuric acid) in an inert organic solvent (e.g. methylene chloride, THF, dioxan or a lower alkanol, at a temperature of from ambient to the reflux temperature of a solvent.

Reaction 6 may be carried out by reaction with anhydrous potassium carbonate or anhydrous sodium carbonate in an absolute alkanol corresponding to the desired group $R^{1'}$ (e.g. absolute methanol or absolute ethanol), generally at a temperature of from -10°C to 100°C.

Reaction 7, when Z^2 is a substituted acyl group (the groups of (2)-(6) in those represented by Z), may be carried out by reaction with a halide or acid anhydride of the carboxylic acid corresponding to Z^2, in the presence of a tertiary amine (e.g. pyridine or triethylamine), in an inert organic solvent (e.g. methylene chloride, dimethylformamide, THF or ethyl acetate) or in the absence of a solvent, at a temperature of from 0°C to 50°C, or when Z^2 represents a group of the formula:



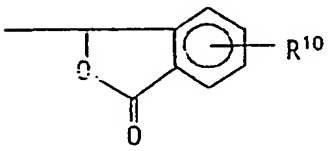
(in which R^{10} is as hereinbefore defined), may be carried out by reaction with a halide of the compound

corresponding to Z^2, in the presence of a base (e.g. a tertiary amine or a hydroxide or carbonate of an alkali metal), in an inert organic solvent (e.g. methylene chloride, dimethylformamide, THF or acetone) at a temperature of from 10°C to 50°C.

Reaction 8 is a desilylation, and may be carried out by using tetrabutylammonium fluoride ($_nBu_4N^+F^-$) in tetrahydrofuran at room temperature.

Reaction 9 is an esterification, and may be carried out by reaction with a diazoalkane (e.g. diazomethane), in an inert organic solvent (e.g. diethyl ether, ethyl acetate, methylene chloride, acetone or a lower alkanol) at a temperature of from -10°C to 40°C, or by reaction with a lower alkanol in the presence of an acid (e.g. hydrochloric acid or p-toluenesulfonic acid) or in the presence of a condensing agent (e.g. dicyclohexylcarbodiimide), at a temperature of from -10°C to 50°C.

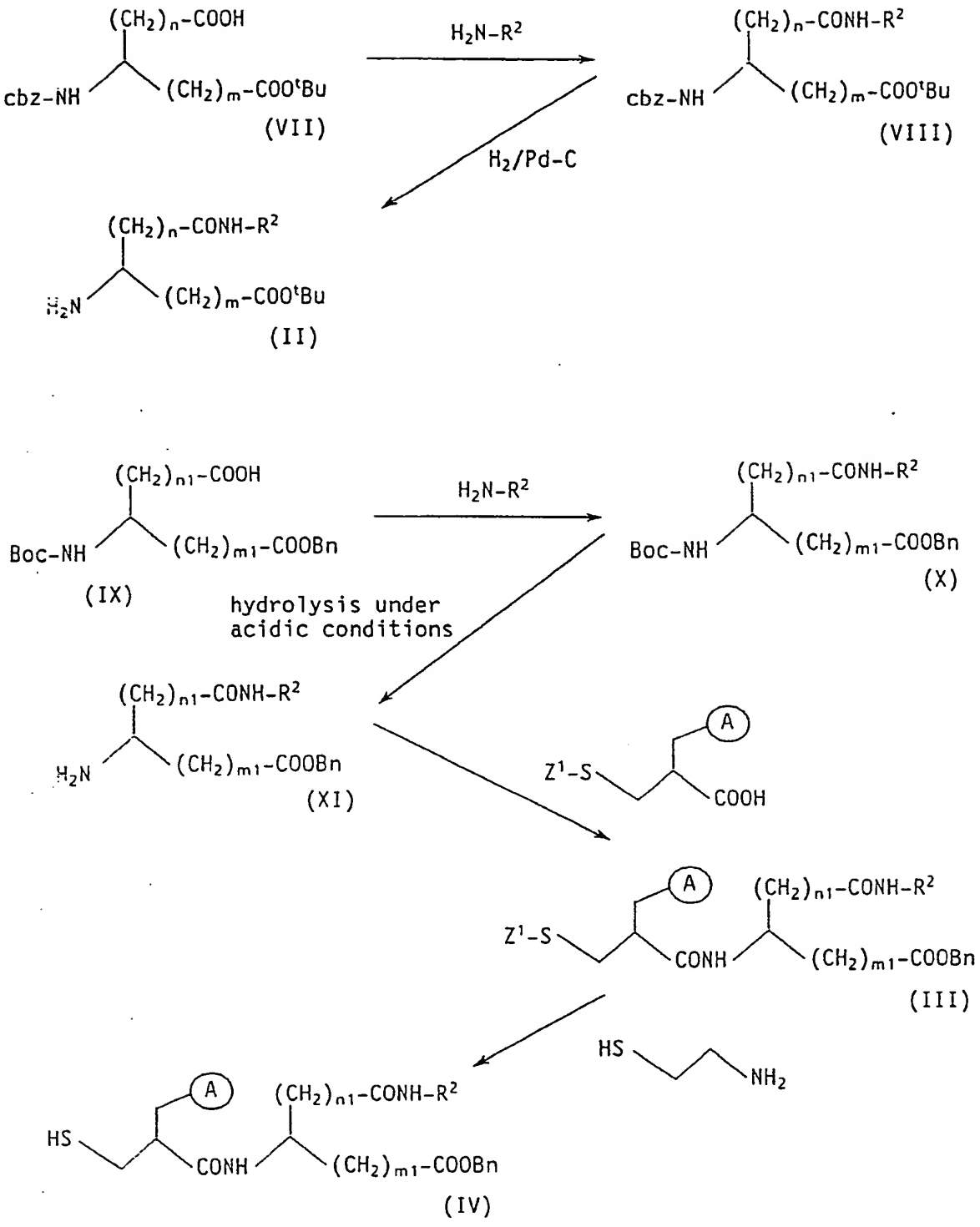
Reaction 10 may be carried out by the same procedure as Reaction 7 when Z^2 represents a group of the formula:



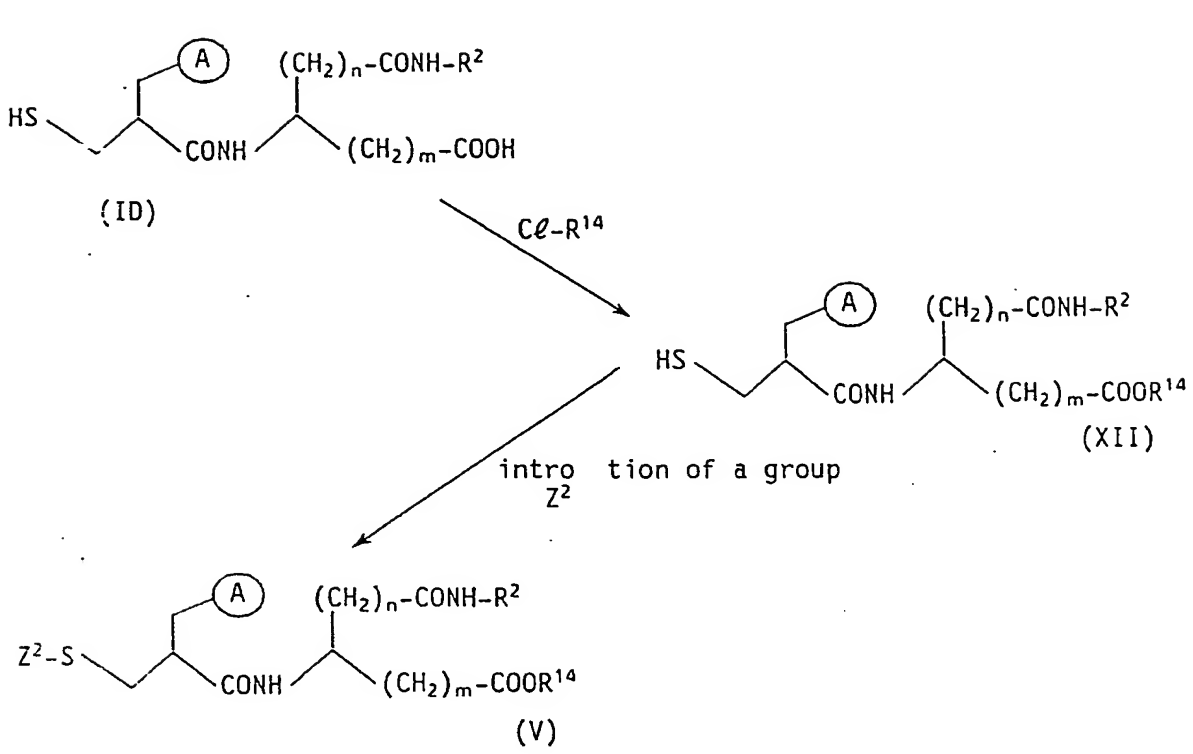
wherein R^{10} is as hereinbefore defined.

The compounds of the general formulae (II), (III), (IV) and (V), used in the aforesaid schemes, may be prepared by the combination of known methods, for example, by using the series of reactions depicted in the following Scheme D, wherein Boc represents a tert-butoxycarbonyl group, cbz represents a benzyloxycarbonyl group and the other symbols are as hereinbefore defined.

Scheme D


(CH2)n-COOH
cbz-NH
(CH2)m-COOtBu
(VII)
H2N-R2
(CH2)n-CONH-R2
cbz-NH
(CH2)m-COOtBu
(VIII)
H2/Pd-C
(CH2)n-CONH-R2
H2N
(CH2)m-COOtBu
(II)
(CH2)n1-COOH
Boc-NH
(CH2)m1-COOBn
(IX)
H2N-R2
(CH2)n1-CONH-R2
Boc-NH
(CH2)m1-COOBn
(X)
hydrolysis under
acidic conditions
(CH2)n1-CONH-R2
H2N
(CH2)m1-COOBn
(XI)
A
Z1-S
COOH
A
(CH2)n1-CONH-R2
Z1-S
CONH
(CH2)m1-COOBn
(III)
HS
NH2
A
(CH2)n1-CONH-R2
HS
CONH
(CH2)m1-COOBn
(IV)

Scheme D (continued)



Each of the steps depicted in the foregoing scheme is well known to those skilled in the art.

Throughout the specification, in each of the reactions, products may be purified by conventional methods, for example, distillation at atmospheric or reduced pressure, high performance liquid chromatography, thin layer chromatography or column chromatography using silica gel or magnesium silicate or washing or recrystallization. Purification may be carried out after each reaction, or after a series of reactions.

Starting materials of the formula (VII) and (IX) and the reagents, used in the preparation of compounds of the present invention, are known compounds per se, or may be easily prepared by known methods.

For example, the compounds of formula (VII), wherein n is zero and m is 1 or 2, of formula (IX), wherein n_1 is zero and m_1 is 1 or 2, and of the formula (IX), wherein m_1 is zero and n_1 is 1 or 2, are commercially available.

The compounds of the general formula (I) and non-toxic salts thereof, of the present invention have an inhibitory effect on enkephalinase, and are, therefore, useful as analgesic, antianxiety or anticonvulsant agents, in mammals, especially humans.

The inhibitory effect on enkephalinase and the analgic effect based on the inhibitory effect, of the compounds of the present invention were confirmed by the screening tests described below.

Inhibitory effect on enkephalinase

(1) Method

Enkephalinase was obtained by the procedure as described in Journal of Neurochemistry, 39, 1081 (1982). That is, to striata obtained from ddY male mice (weighing 28 ~ 30 g), Tris hydrochloric acid buffer solution (referred to as "Tris buffer" hereafter) was added. The mixture was homogenised and centrifuged (1000 g x 5 minutes). The supernatant obtained was further centrifuged (20000 g x 1 minute). The resulting pellet was washed with cold Tris buffer and resuspended in a fresh Tris buffer to use as an enzyme source of enkephalinase.

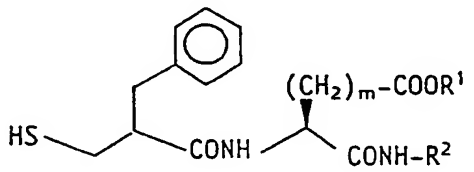
The experiment was carried out according to the method described in Journal of Biological Chemistry, 255, 2227 (1980). That is, the test compounds (10^{-5} ~ 10^{-9} M) were dissolved in 2% DMSO/Tris buffer (50 µl). Incubation at 37°C for 60 minutes was started by adding thereto 150 µg of enzyme solution (x30 dilution of the above enzyme source) and 50 µl of a substrate solution containing succinyl-alanyl-alanyl-phenylalanyl (7-amido-4-methyl)coumarin (i.e. Suc-Ala-Ala-Phe-AMC, final concentration: 10^{-4} M) dissolved in 50 mM

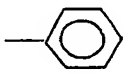
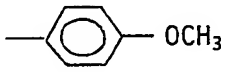
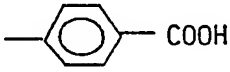
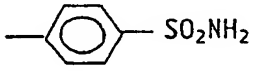
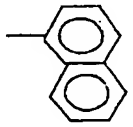
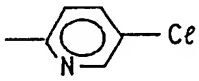
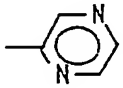
HEPES/NaOH buffer (pH 7.4). The reaction was stopped by the addition of thiorphan (10^{-6} M) and by heating the samples at 95°C for 15 minutes. In a second step, the incubation medium was further incubated at 56°C for 60 minutes in the presence of 0.75 µl of aminopeptidase M. The appearance of AMC fluorescence was measured (exc. 367 nm, em. 440 nm). Blank values were obtained by the same procedure as above described by using thiorphan (10^{-6} M) instead of the test compound.

(2) Result

The results are shown in Table I below.

Table I: Inhibitory effect on enkephalinase (1)



Compounds Example No.	Structure			Inhibitory effect (IC_{50}, M)
	R^1	R^2	m	
1	H		2	2.1×10^{-8}
8(4)	H		1	5.0×10^{-8}
3(2)	H		2	3.6×10^{-9}
3(8)	H		2	2.3×10^{-8}
2(7)	H		2	3.5×10^{-7}
3(4)	H		2	3.5×10^{-8}
3(9)	H		2	1.9×10^{-7}

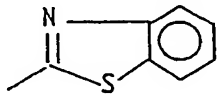
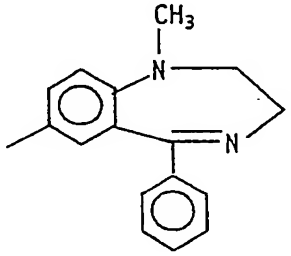
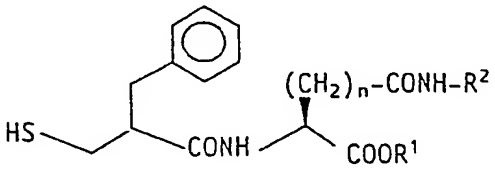
2(10)	H		2	4.4×10^{-8}
4	CH_3		1	1.5×10^{-7}

Table I: Inhibitory effect on enkephalinase (2)



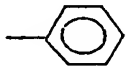
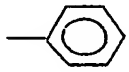
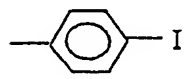
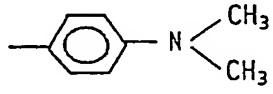
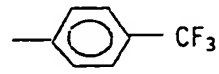
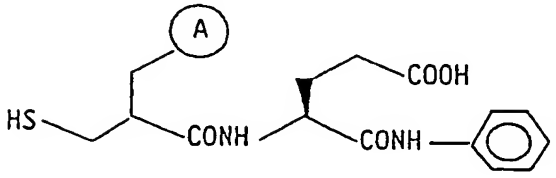
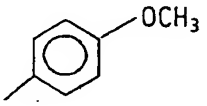
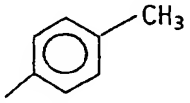
Compounds Example No.	Structure			Inhibitory effect (IC_{50}, M)
	R^1	R^2	n	
12	Na		1	1.3×10^{-8}
11	Na		2	6.8×10^{-8}
12(5)	H		1	2.8×10^{-9}
12(7)	H		1	2.4×10^{-8}
12(8)	H		1	2.1×10^{-8}

Table I: Inhibitory effect on enkephalinase (3)



Compounds Example No.	Structure A	Inhibitory effect (IC_{50}, M)
1(1)		4.0×10^{-7}
1(2)		5.1×10^{-8}
1(3)		7.1×10^{-8}

Inhibitory effect on bradykinin-induced biting-like response

(1) Method

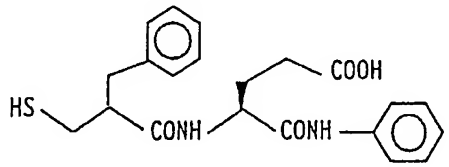
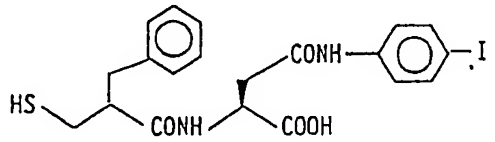
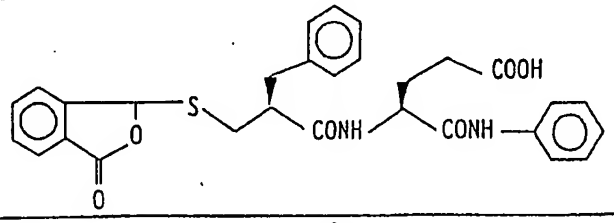
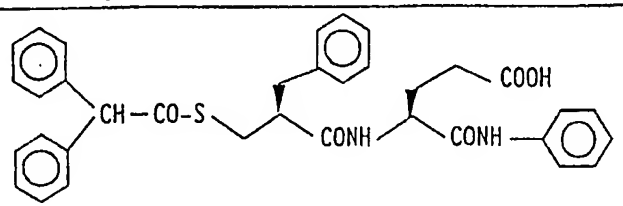
The experiment was carried out according to the method described in Journal of Pharmacological Methods, 7, 271 (1982). That is, male Sprague-Dawley rats (weighing 200 ~ 300 g) were used. Implantation of a bradykinin (0.63 ~ 1.25 µg in 0.5 ~ 1.0 µl of distilled water) - filled cannula onto the tooth pulp and the fixation of it on the lower incisor surfaces were carried out under ethyl ether anesthesia. A micro-application of bradykinin onto the tooth pulp produced biting-like response and some other aversive behavior such as jumping, struggling, rubbing, scratching, escape, head-jerk and body-jerk within 1 minute. Only rats which showed a duration of 20 minutes or more for the biting-like responses before administration of the compound of the present invention, were used for further experiment.

The test compounds suspended in 0.5 % carboxymethyl cellulose solution were administered either intraperitoneally or orally. After regular intervals bradykinin was administered. The number of rats which did not show the biting-like response after adminsitration (i.e. analgic state) was counted.

(2) Result

The results are shown in Table II below.

Table II: Inhibitory effect on bradykinin-induced biting-like response

Compounds Example No.	Structure	Dose (mg/kg)	Number of rats	Number of rats which did not show the biting-like response			
				10 30	40 60	70 90	100 minutes (i.p.) 120 after (p.o.)
1		10 (i.p.)	5	4	4	3	1
12(5)		10 (i.p.)	8	4	2	1	0
16		100 (p.o.)	8	3	6	2	0
13(5)		100 (p.o.)	8	1	7	1	1

EP 0 341 081 A2

The acute toxicity of the compounds of the present invention is very weak. For example, the acute toxicity (LD_{50}) of N-[3-(phthalid-3-yl)thio-2S-benzylpropionyl]- α -anilino-L-glutamic acid is 500-1000 mg/kg by intraperitoneal injection in mice. Therefore, the compounds of the present invention may be considered to be sufficiently safe and suitable for pharmaceutical use.

For the purpose above described, the compounds of the present invention will normally be administered systemically or partially, usually by oral or parenteral administration.

The doses to be administered are determined depending upon, for example, age, body weight, symptom, the desired therapeutic effect, the route of administration, and the duration of the treatment. In the human adult, the doses per person per dose are generally between 10 mg and 1 g, by oral administration, up to several times (preferably 1 to 4 times) per day, and between 1 mg and 100 mg, by parenteral administration (preferably, intravenous administration) up to several times (preferably 1 to 4 times) per day.

As the doses to be used depend upon various factors there will be cases in which doses lower than or greater than the ranges specified above may be used.

The present invention provides a pharmaceutical composition which comprises, as active ingredient, an amino acid derivative of general formula I or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable carrier or coating.

Suitable compositions include solid compositions, liquid compositions and other compositions for oral administration and injections, external compositions and suppositories for parenteral administration.

Solid compositions for oral administration, include compressed tablets, pills, capsules, dispersible powders, and granules. In such compositions, one or more of the active compounds are generally admixed with a least one inert diluent (e.g. lactose, mannitol, glucose, hydroxypropylcellulose, microcrystalline cellulose, starch, polyvinylpyrrolidone or magnesium metasilicate aluminate).

The compositions may also comprise, as is normal practice, additional substances other than inert diluents, e.g. lubricating agents (e.g. magnsium stearate), disintegrating agents (e.g. cellulose calcium glycolate etc.), and agents to assist dissolution (glutamic acid, aspartic acid etc.) and stabilizing agent (e.g. lactose).

The tablets or pills may, if desired, be coated with a film of gastric or enteric material (e.g. sugar, gelatin, hydroxypropylcellulose, hydroxypropylmethyl cellulose phthalate).

Capsules include soft ones and hard ones.

Liquid compositions for oral administration include pharmaceutically-acceptable solutions, emulsions, suspensions, syrups and elixirs.

In such liquid compositions, one or more of the active compounds are admixed with inert diluents commonly used in the art (e.g. purified water, ethanol).

Besides inert diluents, such compositions may also comprise adjuvants such as wetting agents and suspending agents, sweetening agents, flavouring agents, perfuming agents and preserving agents.

Other compositions for oral administration include spray compositions which may be prepared by known methods and which comprise one or more of the active compounds. Spray compositions may comprise additional substances other than inert diluents, e.g. stabilizing agents (e.g. sodium sulfite), isotonic buffer comprising (e.g. sodium chloride, sodium citrate, citric acid). For the preparation of such spray compositions, for example, the method described in the United States Patent No. 2868691 or 3095355 may be used.

Injectable compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions.

In such injectable compositions, one or more of the active compounds are admixed with at least one inert aqueous diluent (e.g. distilled water for injection or physiological salt solution) or inert non-aqueous diluent (e.g. propylene glycol, polyethylene glycol, olive oil, ethanol or POLYSORBATE 80 (registered trade mark).

Injectable compositions may also comprise additional ingredients other than inert diluents, e.g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agents (e.g. lactose), assisting agents such as assisting agents for dissolution (e.g. glutamic acid or aspartic acid).

They may be sterilized by filtration (through a bacteris-retaining filter), incorporation of sterilizing agents in the composition or irradiation. After sterilizing, they can also be converted into sterile solid compositions, for example, by freeze-drying, and thereafter dissolved in sterile water or some other sterile diluents for injection immediately before use.

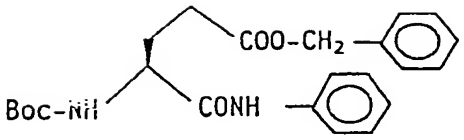
Other compositions for parenteral administration include liquids for external use, and endermic liniments (e.g. ointment), suppositories and pessaries which comprise one or more of the active compounds and may be prepared by known methods.

The following Reference Examples and Examples illustrate the present invention.

The solvents in parentheses show the eluting or developing solvents and the ratios of the solvents used are by volumn in chromatographic separations. Unless otherwise specified. "IR" was measured by KBr method and "NMR" was measured in deuterochloroform ($CDCl_3$) solution.

Reference Example 1

N-(tert-butoxycarbonyl)-α-anilino-L-glutamic γ-benzyl ester



To a solution of N-(tert-butoxycarbonyl)-L-glutamic acid γ-benzyl ester dicyclohexylamine salt (commercially available 5.18g) in methylene chloride (30 ml) was added pivaloyl choride (1.35 ml) under cooling with ice, and the mixture was stirred for 10 minutes at a room temperature. The reaction mixture was again cooled with ice, and then a solution of aniline (1 ml) in triethylamine (1.4 ml) was added dropwise thereto, and the mixture was stirred for 20 minutes at room temperature. The reaction mixture was diluted with ethyl acetate, washed with 1N hydrochloric acid, 1N aqueous solution of sodium hydroxide and a saturated aqueous solution of sodium chloride, successively, dried over magnesium sulfate, and concentrated under reduced pressure.

The residue (solid) was washed with n-hexane to give the title compound (2.9 g) having the following physical data:

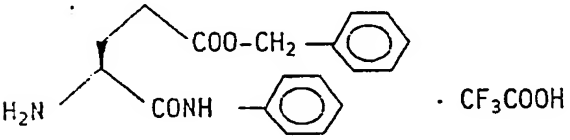
TLC (ethyl acetate : n-hexane = 1:1):Rf 0.74;

NMR: δ 8.40 (1H, brs), 7.55 ~ 6.90 (10H, m), 5.35 (1H, brd), 5.10 (2H, s), 4.40 ~ 4.10 (1H, m), 2.80 ~ 2.40 (2H, m), 2.40 ~ 1.70 (2H, m), 1.42 (9H, s);

MS:m/z 412(M^+), 356, 339, 292.

Reference Example 2

α-anilino-L-glutamic acid γ-benzyl ester trifluoroacetic acid salt



To a solution of glutamic acid protected by a Boc group (2.9g, prepared in Reference Example 1) in methylene chloride (2 ml) was added trifluoroacetic acid (5.4 ml) under cooling with ice, and the mixture was stirred for 2.5 hours at room temperature. The reaction mixture was concentrated under reduced pressure to give the crude title compound (3.00 g) having the following physical data:

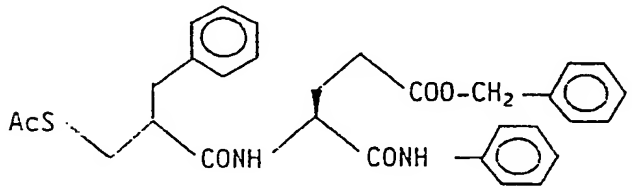
TLC (ethyl acetate) : Rf 0.23;

NMR: δ 9.50(1H, brs), 7.60 ~ 6.80(10H, m), 5.00(2H, s), 4.60 ~ 4.30(1H, m), 2.70 ~ 2.50(2H, m), 2.50 ~ 1.90(2H, m);

MS:m/z 312, 204, 193, 192.

Reference Example 3

N-(3-acetylthio-2RS-benzylpropionyl)-α-anilino-L-glutamic acid γ-benzyl ester



To a solution of 3-acetylthio-2RS-benzylpropionic acid (prepared by the method described hereafter, 833 mg) in methylene chloride (1 ml) was added an excess amount of oxalyl chloride at room temperature, and the mixture was stirred for 30 minutes. Oxalyl chloride was fully distilled off from the reaction mixture, and to the residue was added methylene chloride (2 ml) to obtain a solution of acid chloride.

To a solution of trifluoroacetic acid salt of the amine compound (prepared in Reference Example 2, 1.64 g) in a mixture of pyridine (2.83 ml) and methylene chloride (16 ml), was added dropwise the solution of acid chloride prepared hereinbefore under cooling with ice and the mixture was stirred for 15 minutes at room temperature. The reaction mixture was poured into water, washed with 1N hydrochloric acid, 1N aqueous solution of sodium hydroxide and a saturated aqueous solution of sodium chloride, successively, dried over

magnesium sulfate and concentrated under reduced pressure.

The residue was purified by column chromatography on silica gel (methyene chloride : n-hexane = 2 : 1 → methyiene chloride → methylene chloride → methylene chloride : methanol = 20 : 1) and further recrystallized from a mixture of ethyl acetate and n-hexane to give the title compound (743 mg) as a white powder having the following physical data:

TLC (ethyl acetate : n-hexane = 1 : 1) : Rf 0.48;

NMR: δ 8.52 and 8.38(1H, brs), 7.34 ~ 7.00(15H, m), 6.44 ~ 6.24(1H, m), 5.12 and 5.10(2H, s), 4.60 ~ 4.30(1H, m), 3.24 ~ 3.00 and 3.00 ~ 2.82(4H, m), 2.76 ~ 2.34(3H, m), 2.28 and 2.22(3H, s), 2.20 ~ 1.82(2H, m);

MS:m/z 532(M^+), 489, 440, 370.

3-Acetylthio-2RS-benzylpropionic acid, used as a starting material in a procedure hereinbefore described, was prepared as follows.

To 2RS-benzylacrylic acid (being on the market, 25 g) was added thioacetic acid (16 ml) and the mixture was refluxed for one hour by heating in an oil bath. An excess amount of thioacetic acid was distilled off from the residue and further distilled off as an azeotropic mixture with toluene. The residue was purified by column chromatography on silica gel (ethyl acetate : n-hexane : acetic acid = 5 : 95 : 0.05 → 20 : 80 : 0.05) to give 3-acetylthio-2RS-benzylpropionic acid (16.7 g) as colorless oil having the following physical data:

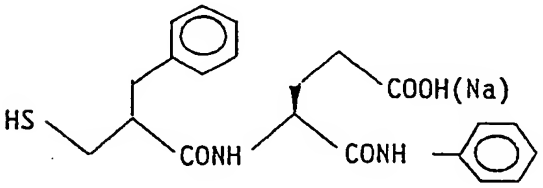
TLC (n-hexane : ethyl acetate + 1 : 1) : Rf 0.03;

MMR: δ 7.35 ~ 7.14(5H, m), 3.2 ~ 2.8(5H, m), 2.33(3H, s) ;

MS:m/z 238(M^+), 220.

Example 1

N-(3-mercapto-2RS-benzylpropionyl)-α-anilino-L-glutamic acid and their γ-sodium salt



Under an atmosphere of argon, to a solution of the benzyl ester compound (prepared in Reference Example 3, 743 mg) in a mixture of tetrahydrofuran (9 ml) and dimethoxyethane (1.4 ml) was added a solution of lithium hydroxide monohydrate (293 mg) in water (5 ml) at room temperature and the mixture was stirred for 30 minutes at the same temperature. To the reaction mixture was added 1N hydrochloric acid under cooling with ice to acidify and the mixture was extracted with ethyl acetate. The extract was washed with a saturated aqueous solution of sodium chloride, dried over sodium sulfate and concentrated under reduced pressure.

The residue was purified by column chromatography on silica gel (chloroform → acetic acid : chloroform = 1 : 99) to give the title compound (387 mg as free acid) as a white powder having the following physical data:

Melting point: 158.5 ~ 160.0 °C;

TLC (acetic acid : chloroform = 5 : 95) : Rf 0.20;

NMR(CDCl$_3$ + DMSO-d$_6$) :

δ 9.04 ~ 8.86(1H, d like m)), 7.56 ~ 7.40(2H, m), 7.36 ~ 6.92(9H, m), 4.72 ~ 4.46(1H, m), 3.02 ~ 2.40 (6H, m), 2.40 ~ 1.68(4H, m), 1.54(1H, t) ;

MS:m/z 400(M^+), 382, 366, 353, 335;

IR(KBr): ν 3275, 1700, 1640, 1600, 1540, 1440, 1300, 1250, 750, 695, cm^{-1}.

The free acid compound obtained above was dissolved in a small amount of methanol and 1N aqueous solution of sodium hydroxide and water were added thereto and the mixture was stirred for 5 minutes at a room temperature. The reaction mixture was lyophilized to give the title compound (as sodium salt) having the following physical data:

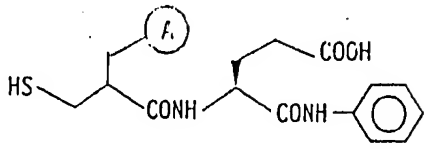
TLC (chloroform : acetic acid = 95 : 5) : Rf 0.20;

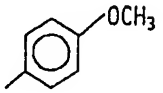
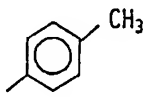
NMR(CD$_3$OD): δ 7.60 ~ 7.00(10H, m)), 4.50 ~ 4.20(1H, m), 3.05 ~ 1.70(9H, m);

IR: ν 3680 ~ 2500(3270) 1635, 1595, 1540, 1440, 1400, 1310, 1250, 750, 695 cm^{-1}.

The desired compounds shown in the following Table III were obtained by the same procedure as a series of reaction of Reference Example 1 → Reference Example 2 → Reference Example 3 → Example 1, by using a corresponding 3-acetylthio-2-substituted propionic acid.

Table III



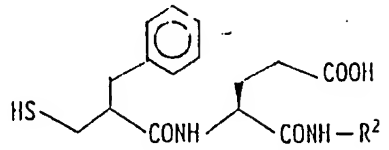
Example No.	A	Chemical name	TLC	IR (ν, cm^{-1})
1(1)		N-(3-mercapto-2RS-cyclohexylmethyl-propionyl)-α-anilino-L-glutamic acid	Rf 0.33 (acetic acid: chloroform = 5:95)	3600 ~ 2400, 1710, 1640, 1540, 1440, 1245, 755
1(2)		N-[3-mercapto-2RS-(4-methoxybenzyl) -propionyl]-α-anilino-L-glutamic acid	Rf 0.40 (acetic acid: chloroform = 1:9)	3650 ~ 2250, (3270, 3040, 2920), 1700 ~ 1600(1630), 1505, 1440, 1295, 1240, 1175, 1115, 750, 690
1(3)		N-[3-mercapto-2RS-(4-methylbenzyl)-propionyl]-α-anilino-L-glutamic acid	Rf 0.47 (acetic acid: chloroform = 1:9)	3650 ~ 2200(3270, 3040, 2925), 1705, 1640, 1600, 1540, 1440, 1305, 1250, 755

EP 0 341 081 A2

Example 2

The desired compounds shown in the following Table IV were obtained by the same procedure as a series of reactions of Reference Example 1 → Reference Example 2 (hydrochloric acid instead of trifluoroacetic acid was used) → Reference Example 3 → Example 1, by using corresponding starting material and the amine compound.

Table IV



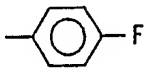
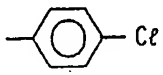
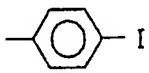
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1}) or Melting point
2(1)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-fluoroanilino)-L-glutamic acid	Rf 0.19 (acetic acid: chloroform = 8:92)	3275, 1705, 1640, 1540, 1505, 1440, 1405
2(2)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-chloroanilino)-L-glutamic acid	Rf 0.28 (acetic acid: chloroform = 8:92)	3275, 1705, 1640, 1600, 1530, 1495
2(3)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-iodoanilino)-L-glutamic acid	Rf 0.34 (acetic acid: chloroform = 8:92)	181.0 ~ 185.0 °C

Table IV (continued)

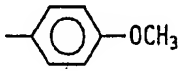
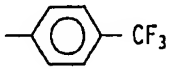
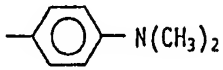
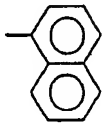
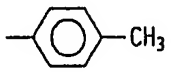
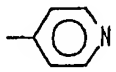
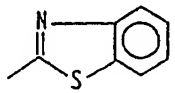
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
2(4)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-methoxyanilino)-L-glutamic acid	Rf 0.25 (acetic acid: chloroform = 2:8)	3275, 1700, 1640, 1510, 1440, 1420, 1300, 1240
2(5)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-trifluoromethylanilino)-L-glutamic acid	Rf 0.28 (acetic acid: chloroform = 5:95)	3275, 1700, 1690, 1640, 1610, 1520, 1410
2(6)		N-(3-mercapto-2RS-benzylpropionyl)-α-[4-(N,N-dimethylamino)anilino]-L-glutamic acid	Rf 0.43 (chloroform: methanol: acetic acid = 30:3:1)	3275, 1710, 1650, 1640, 1520, 1450
2(7)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(1-naphthyl)amide	Rf 0.25 (acetic acid: chloroform = 5:95)	3625 ~ 2300(3260, 3025), 1695, 1630, 1525, 1500, 1430, 1395, 1265, 790, 770, 695

Table IV (continued)

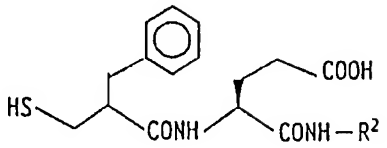
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
2(8)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-methylanilino)-L-glutamic acid	Rf 0.29 (acetic acid: chloroform = 8:92)	3275, 1710, 1660, 1640, 1610, 1540, 1530, 1510, 1440, 1410
2(9)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(4-pyridyl)amide	Rf 0.18 (chloroform: methanol: acetic acid = 30:5:1)	3250, 1720, 1700, 1630, 1590, 1500
2(10)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(2-benzthiazolyl) amide	Rf 0.52 (chloroform: methanol: acetic acid = 30:3:1)	3650 ~ 2150(3275, 3050, 2925), 1700, 1640, 1530, 1440, 1305, 1285, 750, 700

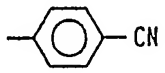
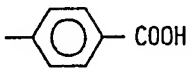
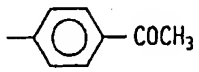
EP 0 341 081 A2

Example 3

The desired compounds shown in the following Table V and VI were obtained by the same procedure as a series of reactions of Reference Example 1 (the amino bond-forming reaction using dicyclohexylcarbodiimide (DCC) as a condensing agent, was used instead of the method using a mixed acid anhydride with pivaloyl chloride) → Reference Example 20 (hydrochloric acid instead of trifluoroacetic acid was used) → Reference Example 3 → Example 1, by using corresponding starting material and the amine compound.

Table V



Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
3(1)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-cyanoanilino)-L-glutamic acid	Rf 0.36 (acetic acid: chloroform = 1:9)	3650 ~ 2320(3300), 2240, 1690, 1640, 1590, 1410, 1310, 1255, 1180, 840, 755, 700, 555
3(2)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-carboxyanilino)-L-glutamic acid	Rf 0.39 (chloroform: methanol: acetic acid = 30:3:1)	3700 ~ 2340(3270, 3050), 1690, 1680, 1640, 1595, 1525, 1410, 1250, 1175, 1005, 855, 775, 700
3(3)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-acetylanilino)-L-glutamic acid	Rf 0.34 (acetic acid: chloroform = 1:9)	3670 ~ 2330(3350), 1710, 1640, 1595, 1525, 1405, 1360, 1320, 1270, 1180, 840, 700

EP 0 341 081 A2

Table V (continued)

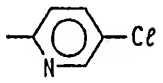
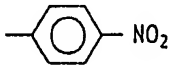
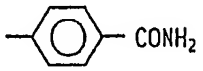
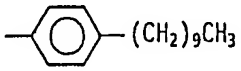
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
3(4)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(5-chloropyridin-2-yl)amide	Rf 0.41 (acetic acid: chloroform = 5:95)	3640 ~ 2200(3300, 3050), 1705, 1640, 1580, 1520, 1460, 1380, 1305, 1115, 700
3(5)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-nitroanilino)-L-glutamic acid	Rf 0.17 (acetic acid: chloroform = 4:96)	3300, 1710, 1645, 1620, 1600, 1500, 1450, 1415, 1340
3(6)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-carbamoylanilino)-L-glutamic acid	Rf 0.38 (acetic acid: ethyl acetate = 5:95)	3300, 1710 (shoulder), 1700 ~ 1660 (shoulder), 1640, 1605, 1520, 1410
3(7)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-decylanilino)-L-glutamic acid	Rf 0.46 (acetic acid: chloroform = 5:95)	3280, 2940, 2860, 1700, 1680, 1600, 1515, 1445, 1410 1255, 700

Table V (continued)

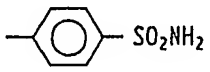
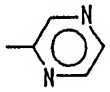
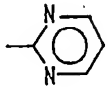
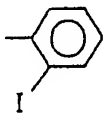
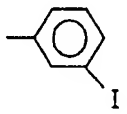
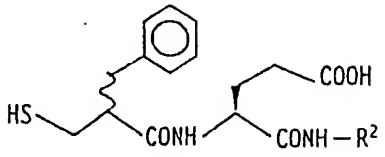
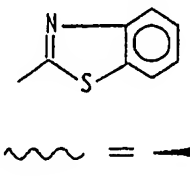
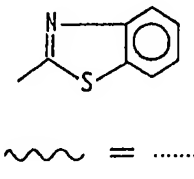
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
3(8)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-sulfamoylanilino)-L-glutamic acid	Rf 0.52 (acetic acid: ethyl acetate = 5:95)	3300, 1700 (shoulder), 1645, 1595, 1520, 1400, 1330, 1255, 1160
3(9)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(pyrazin-2-yl) amide	Rf 0.26 (acetic acid: chloroform = 1:9)	3650 ~ 2240(3275, 3040, 2930), 1705, 1630, 1530, 1410, 1300, 1270, 1210, 1060, 1010, 845, 750, 700
3(10)		N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid α-(pyrimidin-2-yl) amide	Rf 0.26 (chloroform: methanol: acetic acid = 30;3:1)	3250, 1710, 1650, 1580, 1510, 1440, 1415
3(11)		N-(3-mercapto-2RS-benzylpropionyl)-α-(2-iodoanilino)-L-glutamic acid	Rf 0.24 (acetic acid: chloroform = 5:95)	3250, 3025, 1700, 1630, 1575, 1515, 1430, 1280, 1010, 750, 700

Table V (continued)

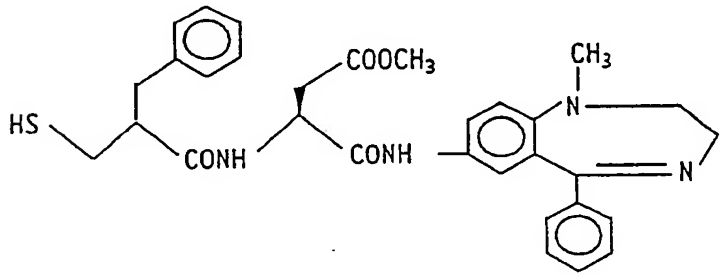
Example No.	R^2	Chemical name	TLC	IR (ν, cm^{-1})
3(12)		N-(3-mercapto-2RS-benzylpropionyl)-α-(3-iodoanilino)-L-glutamic acid	Rf 0.24 (acetic acid: chloroform = 5:95)	3260, 3030, 1705, 1635, 1580, 1520, 1470, 1410, 1300, 1240, 865, 780, 750, 700, 680



Example No.	R^2	Chemical name	TLC	Optical rotation ($[\alpha]_D$)
3(13)		N-(3-mercapto-2S-benzylpropionyl)-L-glutamic acid α-(2-benzthiazolyl) amide	Rf 0.25 (acetic acid: chloroform = 5:95)	+ 9.83° ($CHC\ell_3$, c=1.005)
3(14)		N-(3-mercapto-2R-benzylpropionyl)-L-glutamic acid α-(2-benzthiazolyl) amide	Rf 0.29 (chloroform: tetrahydrofuran: acetic acid = 30:8:1)	- 35.6° ($CHC\ell_3$, c=1.00)

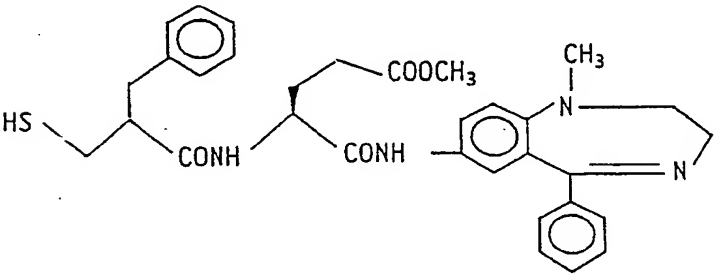
Example 4

N-(3-mercapto-2RS-benzylpropionyl)-L-aspartic acid
α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide β-methyl ester



To a solution of N-(3-acetylthio-2RS-benzylpropionyl)-L-aspartic acid α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl) amide β-benzyl ester (prepared by the same procedure as a series of reactions of Reference Example 1 → Reference Example 2 → Reference Example 3, by using the corresponding starting materials 1,686 g) in methanol (10 ml) was added potassium carbonate (0.688 g) and the mixture was stirred for one hour at room temperature. The reaction mixture was diluted with methylene chloride (100 ml), washed with water and a saturated aqueous solution of sodium chloride, dried over magnesium sulfate and concentrated under reduced pressure. the residue was purified by column chromatography on silica gel (methylene chloride : methanol = 30 : 1) to give the title compund (0.735 g) as orange amorphous having the following physical data:
TLC (methylene chloride : methanol = 95 : 5) : Rf 0.37:
NMR: δ 8.32 and 7.98(1H, s), 7.68 ~ 6.64(14H, m), 4.90 ~ 4.75(1H, m), 3.88 ~ 3.40(7H, m), 3.20 ~ 1.92(10H, m), 1.40(1H, 2xt);
MS:m/z 558(M^+), 526, 510, 498:
IR(KBr) : ν 3600 ~ 2500, 1715, 1650, 1610, 1490, 1290, 1180, 690. The following desired compound was obtained by the same procedure as above.

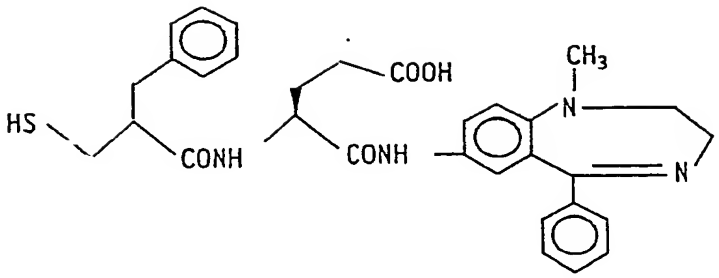
(1) N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid
α-(2,3-dihydro1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide γ-methyl ester



TLC (methanol : methylene chloride = 1 : 9) : Rf 0.56;
NMR: δ 8.48 and 8.27(1H, s, 5 : 6), 7.70 ~ 6.90(13H, m), 6.70 and 6.52(1H, d), 4.55 ~ 4.28(1H, m), 3.80 ~ 3.50(4H, m), 3.63 and 3.65(3H, s), 3.00 ~ 1.84(9H, m), 2.80 and 2.76(3H, s), 1.44 and 1.39(1H, t);
MS:m/z 572(M^+), 540, 512;
IR:ν 3300, 1740, 1650, 1500.

Example 5

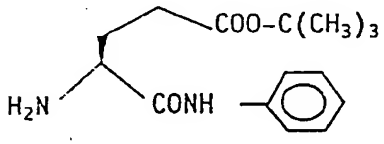
N-(3-mercapto-2RS-benzylpropionyl)-L-glutamic acid
α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide



The title compound having the following physical data was obtained by the same procedure as Example 1, by using the methyl ester prepared in Example 4(1).
TLC (methanol : benzene = 2 : 8) : Rf 0.2 ;
Melting point : 126.0 ~ 131.0 °C

Reference Example 4

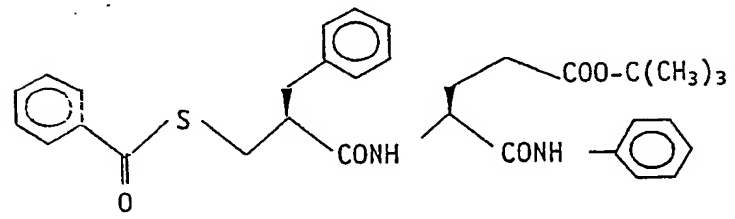
α-anilino-L-glutamic acid γ-tert-butyl ester



Under an atmosphere of hydrogen, a mixture of N-(benzyloxycarbonyl)-α-anilino-L-glutamic acid γ-tert-butyl ester (7.80 g, prepared by the same procedure as Reference Example 1 (the amido bond forming reaction using DCC as a condensing agent, was used instead of the method using a mixed acid anhydride) by using N-(benzyloxycarbonyl)-L-glutamic acid γ-tert-butyl ester as starting material), palladium-carbon (10% ; 880 mg) and ethyl acetate (130 ml) was stirred for 3 hours at room temperature. The reaction mixture was filtered, and to the filtrate was added ethyl acetate (20 ml) containing 4N hydrogen chloride, and then the mixture was concentrated under reduced pressure to give hydrochloric acid salt of the title compound as crude product.

Example 6

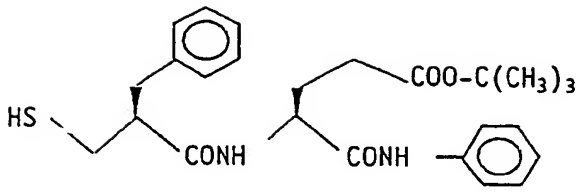
N-(3-benzoylthio-2S-benzylpropionyl)-α-anilino-L-glutamic acid γ-tert-butyl ester



The title compound having the following physical data was obtained by the same procedure as Reference Example 3, by using the amine compound prepared in Reference Example 4 and 3-benzoylthio-2S-benzylpropionic acid.
TLC (n-hexane : ethyl acetate = 1 : 1) : Rf 0.56.

Example 7

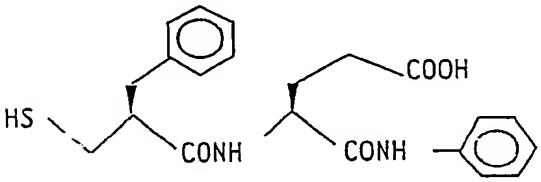
(N-(3-mercapto-2S-benzylpropionyl)-α-anilino-L-glutamic acid γ-tert-butyl ester



The title compound having the following physical data was obtained by the same procedure as Example 4, by using the benzoyl compound prepared in Example 6
TLC (n-hexane : ethyl acetate = 1 : 1) : Rf 0.53.

Example 8

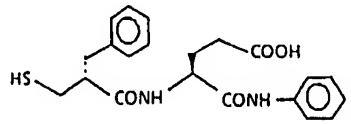
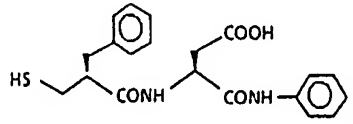
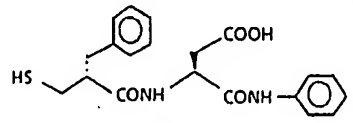
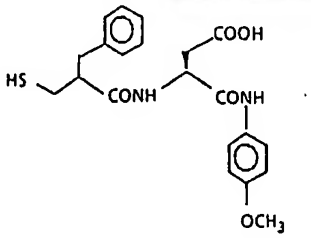
N-(3-mercapto-2S-benzylpropionyl)-α-anilino-L-glutamic acid



To a solution of the tert-butyl ester (770 mg) prepared in Example 7, in ethyl acetate (3 ml) was added ethyl acetate (2 ml) containing 4N hydrogen chloride, and the mixture was stirred for two hours at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (methylene chloride : ethyl acetate = 9 : 1 → 4 : 1 → 2 : 1 → ethyl acetate) to give the title compound (300 mg) having the following physical data:
TLC (chloroform : tetrahydrofuran : acetic acid = 15 : 4 : 1) : Rf 0.39;
Optical rotation (c = 0.895, absolute C_2H_2OH) : $[\alpha]_D$-22.2°.

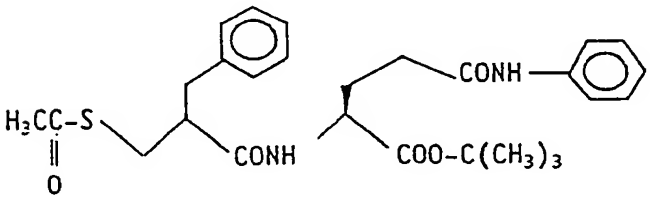
The desired compounds shown in the following Table VII were obtained by the same procedure as a series of reaction of Reference Example 4 → Example 6 → Example 7 → Example 8, by using corresponding starting materials.

Table VII

Example No.	Structure	Chemical name	TLC	Optical rotation ($[\alpha]_D$) or IR (ν, cm^{-1})
8(1)		N-(3-mercapto-2R-benzylpropionyl)-α-anilino-L-glutamic acid	Rf 0.30 (acetic acid: chloroform = 5:95)	$[\alpha]_D$ -91.72° (c=1.01, absolute C_2H_5OH)
8(2)		N-(3-mercapto-2S-benzylpropionyl)-α-anilino-L-aspartic acid	Rf 0.425 (chloroform: tetrahydrofuran: acetic acid = 15:4:1)	$[\alpha]_D$ -28.64° (c=1.00, CH_3OH))
8(3)		N-(3-mercapto-2R-benzylpropionyl)-α-anilino-L-aspartic acid	Rf 0.46 (acetic acid: chloroform = 5:95) (twice developing)	$[\alpha]_D$ -86.0° (c=0.82, CH_3OH)
8(4)		N-(3-mercapto-2RS-benzylpropionyl)-α-(4-methoxyanilino)-L-aspartic acid (less polar isomer)	Rf 0.44 (chloroform: tetrahydrofuran: acetic acid = 15:4:1)	3290, 3050, 2930, 1705, 1645, 1515, 1410, 1250, 1170, 1030, 825, 700

Example 9

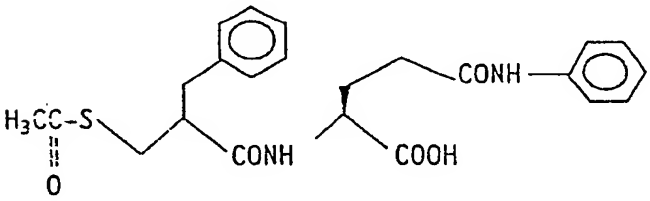
N-(3-acetylthio-2RS-benzylpropionyl)-γ-anilino-L-glutamic acid α-tert-butyl ester



The title compound having the following physical data was obtained by the same procedure as a series of reactions of Reference Example 1 → Reference Example 4 → Reference Example 3, by using N-(benzyloxycarbonyl)-L-glutamic acid α-tert-butyl ester as starting material.
TLC (ethyl acetate : n-hexane = 1 : 1) : Rf 0.42.

Example 10

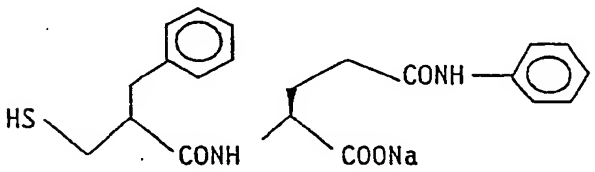
N-(3-acetylthio-2RS-benzylpropionyl)-γ-anilino-L-glutamic acid



The title compound having the following physical data was obtained by the same procedure as Reference Example 2, by using the tert-butyl ester prepared in Example 9 as starting material.
TLC (ethyl acetate : methanol = 9 : 1) : Rf 0.12.

Example 11

N-(3-mercapto-2RS-benzylpropionyl)-γ-anilino-L-glutamic acid α-sodium salt

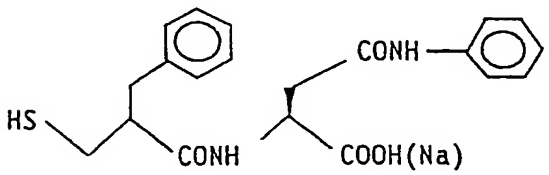


To the acetyl compound (150 mg, prepared in Example 10) in a mixture of chloroform (1 ml) and acetonitrile (2 ml) was added cysteamine (i.e. H_2N-$(CH_2)_2$-SH, 52.2 mg), and the mixture was stirred for 20 minutes at 40°C. The reaction mixture was diluted with ethyl acetate, washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate : n-hexane = 1 : 1) to give the free acid corresponding to the title compound.

The obtained free acid compound was dissolved in a small amount of methanol and 1N aqueous solution of sodium hydroxide and water were added thereto. The mixture was stirred for 5 minutes at room temperture. The reaction mixture was lyophilized to give the title compound (sodium salt) having the following physical data:
Melting point: 131.0 ~ 135°C ;
TLC (chloroform : tetrahydrofuran : acetic acid = 80 : 15 : 5) : Rf 0.23.

Example 12

N-(3-mercapto-2RS-benzylpropionyl)-β-anilino-L-aspartic acid and the corresponding α-sodium salt



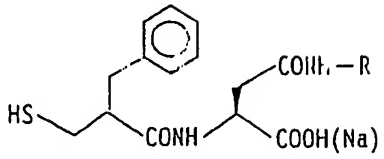
The title compound having the following physical data was obtained by the same procedure as a series of reactions of Reference Example 1 → Reference Example 2 → Reference Example 3 → Example 11, by using N-(tert butoxycarbonyl)-L-aspartic acid α-benzyl ester as starting material.

(1) free acid
TLC (chloroform : tetrahydrofuran : acetic acid = 15 : 4 : 1) : Rf 0.26 ;
IR: ν 3600 ~ 2300, 1725, 1650, 1600, 1530, 1440, 755, 694 cm^{-1}.

(2) sodium salt
TLC (methylene chloride: tetrahydrofuran : acetic acid = 15 : 4 : 1) : Rf 0.35;
IR: ν 3640 ~ 2400, 1650, 1630, 1595, 1530, 1390, 1380 cm^{-1}.

The desired compounds shown in the following Table VIII were obtained by the same procedure as Example 12 by using corresponding starting maerials.

Table VIII



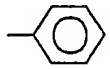
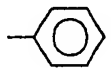
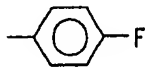
Example No.	R	Chemical name	TLC	Optical rotation ($[\alpha]_D$) or IR (ν, cm^{-1})
12(1)	 (Na salt)	N-(3-mercapto-2RS-benzylpropionyl)-β-anilino-L-aspartic acid α-sodium salt (more polar isomer)	Rf 0.31 (chloroform: tetrahydrofuran: acetic acid = 15:4:1)	$[\alpha]_D$ +22.2° (c=1.53, absolute C_2H_5OH) (as free acid)
12(2)	 (Na salt)	N-(3-mercapto-2RS-benzylpropionyl)-β-anilino-L-aspartic acid α-sodium salt (less polar isomer)	Rf 0.30 (chloroform: tetrahydrofuran: acetic acid = 15:4:1)	$[\alpha]_D$ -6.73° (c=1.54, absolute C_2H_5OH) (as free acid)
12(3)		N-(3-mercapto-2RS-benzylpropionyl)-β-(4-fluoroanilino)-L-aspartic acid	Rf 0.36 (chloroform: methanol: acetic acid = 30:5:1)	3650 ~ 2200(3280), 1715, 1650, 1525, 1500, 1405, 1210, 825

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Table VIII (continued)

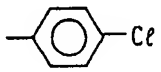
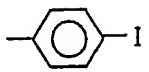
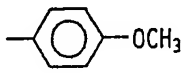
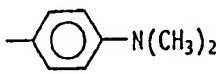
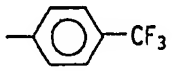
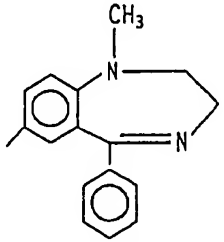
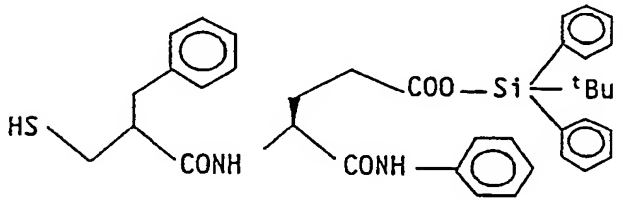
Example No.	R	Chemical name	TLC	Optical rotation ($[\alpha]_D$) or IR (ν, cm^{-1})
12(4)		N-(3-mercapto-2RS-benzylpropionyl)-β-(4-chloroanilino)-L-aspartic acid	Rf 0.38 (chloroform: methanol: acetic acid = 30:5:1)	3645 ~ 2200(3290), 1715, 1655, 1600, 1530, 1490, 1400
12(5)		N-(3-mercapto-2RS-benzylpropionyl)-β-(4-iodoanilino)-L-aspartic acid	Rf 0.40 (chloroform: methanol: acetic acid = 30:5:1)	3650 ~ 2200(3280), 1720, 1650, 1590, 1515, 1480, 1400, 810
12(6)		N-(3-mercapto-2RS-benzylpropionyl)-β-(4-methoxyanilino)-L-aspartic acid	Rf 0.38 (chloroform: methanol: acetic acid = 30:3:1)	3650 ~ 2700, 1640, 1600, 1505, 1410, 1245, 1025, 825
12(7)		N-(3-mercapto-2RS-benzylpropionyl)-β-[4-(N,N-dimethylamino)anilino]-L-aspartic acid	Rf 0.23 (chloroform: methanol: acetic acid = 30:3:1)	3650 ~ 2200(3280), 1650, 1600, 1510, 1310, 820, 700

Table VIII (continued)

Example No.	R	Chemical name	TLC	Optical rotation ($[\alpha]_D$) or IR (ν, cm^{-1})
12(8)		N-(3-mercapto-2RS-benzylpropionyl)-β-(4-trifluoromethylanilino)-L-aspartic acid	Rf 0.33 (chloroform: methanol: acetic acid = 30:3:1)	3670 ~ 2250(3280, 3050, 2920), 1720, 1650, 1605, 1525, 1405, 1320, 1155, 1110, 1065, 840
12(9)		N-(3-mercapto-2RS-benzylpropionyl)-L-aspartic acid β-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide	Rf 0.15 (methylene chloride: methanol: acetic acid = 16:3:1)	3650 ~ 2400, 1640, 1610, 1520, 1490, 1395, 1295

Reference Example 5

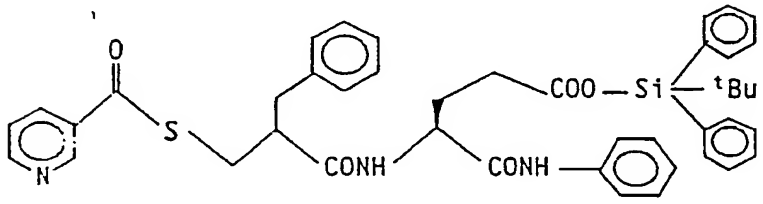
N-(3-mercapto-2RS-benzylpropionyl)-α-anilino-L-glutamic acid γ-tert-butyl-diphenylsilyl ester



To a solution of the carboxylic acid (1.69 g, prepared in Example 1) in dimethylformamide (12 ml) were added imidazole (0.631 g) and successively tert-butyldiphenylsilyl chloride (1.23 ml) at room temperature, and the mixture was stirred for 3 hours at the same temperature. The reaction mixture was dissolved in ether (150 ml), washed with 1N hydrochloric acid, 1N aqueous solution of sodium hydroxide and water, successively, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate: methylene chloride = 5 : 95 → 1 : 9) to give the title compound (2.37 g) as a white powder having the following physical data:
TLC (ethyl acetate : methylene chloride : 1 : 9) : Rf 0.48 ;
NMR: δ 8.58 and 8.46(1H, s × 2), 7.85 ~ 6.98(20H, m), 6.84 ~ 6.62(1H, d × 2), 4.56 ~ 4.39(1H, m), 2.98 ~ 1.02(19H, m);
MS:m/z 638(M^+), 581.

Reference Example 6

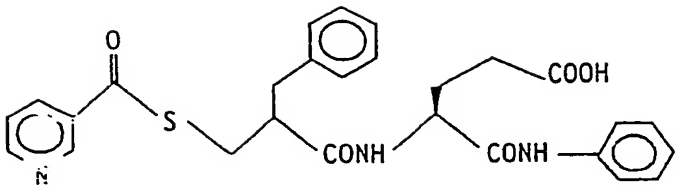
N-(3-nicotinoylthio-2RS-benzylpropionyl)-α-anilino-L-glutamic acid γ-tert-butyl-diphenylsilyl ester



To a mixture of the mercapto compound (1.43 g, prepared in Reference Example 5), nicotinic acid (0.276 g) and diphenylphosphoryl azide (0.99 ml) and dimethylformamide (5 ml), was added triethylamine (0.63 ml) at 0°C and the mixture was stirred for 3 hours at room temperature. To the reaction mixture was added water (30 ml) and the mixture was extracted with ethyl acetate (80 ml × 2). The extract was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (methylene chloride : ethyl acetate = 3 : 1 → 2 : 1) to give the title compound (0.35 g) as a white powder having the following physical data:
TLC (methylene chloride : ethyl acetate = 2 : 1) : Rf 0.44 and 0.38;
NMR: δ 9.12 and 9.02(1H, dd × 2), 8.73 and 8.70 (1H, dd × 2), 8.53 and 8.45(1H, s × 2), 8.13 and 7.95(1H, ddd × 2), 7.75 ~ 7.00(21H, m), 6.60 ~ 6.42(1H, d × 2), 4.52 ~ 4.37(1H, m), 3.45 ~ 1.50(9H, m), 1.11 ~ 1.08(9H, s × 2) ;
MS:m/z 685, 547.

Reference Example 13

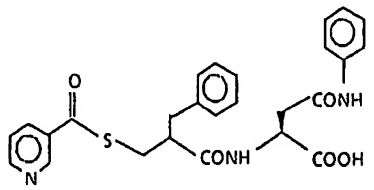
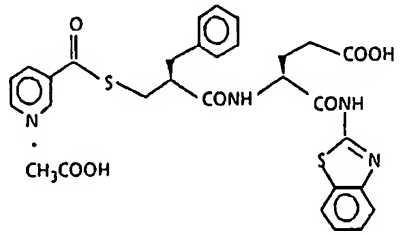
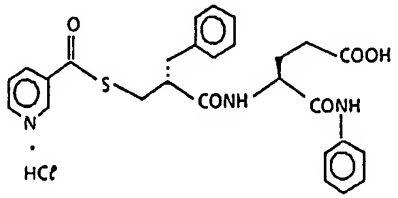
N-(3-nicotinoylthio-2RS-benzylpropionyl)-α-anilino-L-glutamic acid



A solution of the ester compound (350 mg, prepared in Reference Example 6) in a mixture of acetic acid (3 ml), tetrahydrofuran (1 ml) and water (1 ml) was stirred for 16 hours at room temperature, and the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (chloroform : methanol : acetic acid = 80 : 3 : 1 → 50 : 3 : 1) to give the title compound (175 mg) as a white powder having the following physical data:
TLC (chloroform : methanol : acetic acid = 50 : 3 : 1) : Rf 0.47;
NMR($CDCl_3$ + DMSO - d_6) :
δ 8.69 and 9.62(1H, s × 2), 9.05 and 9.00 (1H, d × 2), 8.77 and 8.73(1H, dd × 2), 8.34 ~ 8.04(2H, m), 7.60 ~ 6.90(13H, m), 4.62 ~ 4.36(1H, m), 3.80 ~1.60(9H, m);
MS:m/z 487, 348, 335;
IR(KBr): ν 3600 ~ 2100 (3370, 3040, 2925), 1705, 1640, 1635, 1590, 1525, 1440, 1210, 915, 690 cm^{-1}.

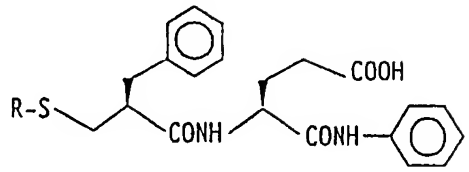
The desired compounds shown in the following Table IX and X were obtained by the same procedure as a series of reactions of Reference Example 5 → Reference Example 6 → Example 13, by using corresponding starting materials.

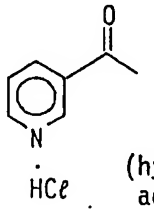
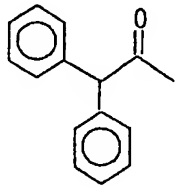
Table IX

Example No.	Structure	Chemical name	TLC	Optical rotation ($[\alpha]_D$) or IR (ν, cm^{-1})
13(1)		N-(3-nicotinoylthio-2RS-benzylpropionyl)-β-anilino-L-aspartic acid	Rf 0.37 (chloroform methanol: acetic acid = 30:5:1)	3650 ~ 2200(3275, 3030), 1715, 1660, 1595, 1530, 1435, 1200, 910, 755, 690
13(2)	 (acetic acid salt)	N-(3-nicotinoylthio-2S-benzylpropionyl)-L-glutamic acid α-(2-benzthiazolyl)amide·acetic acid salt	Rf 0.17 (acetic acid: chloroform = 5:95)	$[\alpha]_D$ -38.6° (c=0.975, tetrahydrofuran)
13(3)	 (hydrochloric acid salt)	N-(3-nicotinoylthio-2R-benzylpropionyl)-α-anilino-L-glutamic acid·hydrochloric acid salt	Rf 0.53 (methylene chloride: tetrahydrofuran: acetic acid = 7:2:1)	3270, 1705 (shoulder), 1665, 1598, 1530, 1442, 1214, 930, 760, 702

EP 0 341 081 A2

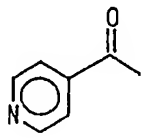
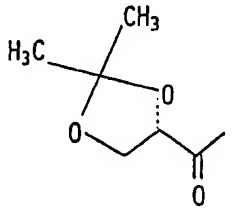
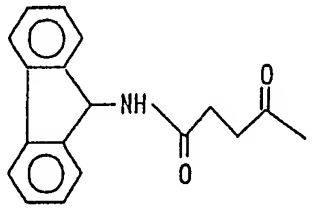
Table X



Example No.	R	Chemical name	TLC	Optical rotation
13(4)	 (hydrochoric acid salt)	N-(3-nicotinoylthio-2S-benzylpropionyl)-α-anilino-L-glutamic acid·hydrochloric acid salt	Rf 0.32 (acetic acid: chloroform = 10:90)	$[\alpha]_D$ -58.6° (c=1.055, CH_3OH)
13(5)		N-(3-diphenylacetylthio-2S-benzylpropionyl)-α-anilino-L-glutamic acid	Rf 0.40 (methanol: chloroform = 10:90)	$[\alpha]_D$ -52.9° (c=1.035, $CHCl_3$,)

EP 0 341 081 A2

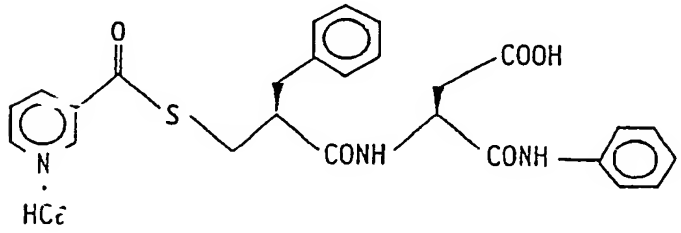
Table X (continued)

Example No.	R	Chemical name	TLC	Optical rotation
13(6)		N-(3-isonicotinoylthio-2S-benzylpropionyl)-α-anilino-L-glutamic acid	Rf 0.36 (chloroform: tetrahydrofuran: acetic acid = 15:4:1)	$[\alpha]_D$ -77.48° (c=1.00, CH_3OH)
13(7)		N-[3-(2,2-dimethyl-1,3-dioxolan-4S-carbonyl)thio-2S-benzylpropionyl]-α-anilino-L-glutamic acid	Rf 0.25 (ethyl acetate)	$[\alpha]_D$ -58.80° (c=1.085, $CHC\ell_3$)
13(8)		N-[3-[N-(9-fluorenyl)succinamoyl] thio-2S-benzylpropionyl]-α-anilino-L-glutamic acid	Rf 0.42 (actic acid: chloroform: = 2:98)	$[\alpha]_D$ -127.5° (c=1.00, dimethyl sulfoxide)

EP 0 341 081 A2

Example 14

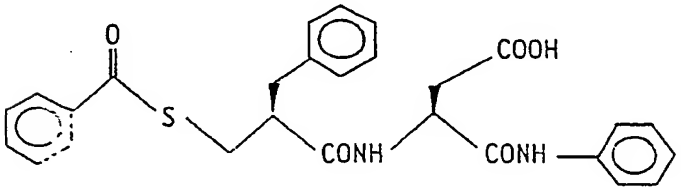
N-(3-nicotinoylthio-2S-benzylpropionyl)-α-anilino-L-aspartic acid hydrochloride



The title compound having the following physical data was obtained by the same procedure as a series of reactions of Reference Example 1 (the amido bond forming reaction using DCC as a condensing agent, was used instead of the method using a mixed acid anhydride) → Reference Example 4 → Example 6 → Example 7 → Example 6 → Example 8, by using N-(benzyloxycarbonyl)-L-aspartic acid β-tert-butyl ester dicyclohexylamine salt as starting material.
TLC (methylene chloride : tetrahydrofuran : acetic acid = 7 : 2 : 1) : Rf 0.56 ;
Optical rotation (c = 1.00, CH_3OH) : $[\alpha]_D$ - 66.22°.

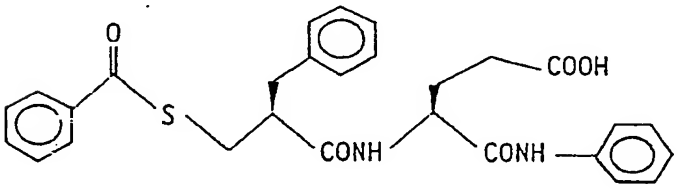
Example 15

N-(3-benzoylthio-2S-benzylpropionyl)-α-anilino-L-aspartic acid



The title compound having the following physical data was obtained by the same procedure as a series of reactions of Reference Example 1 (the amido bond forming reaction using DCC as a condensing agent, was used instead of the method using a mixed acid anhydride) → Reference Example 4 → Example 6 → Example 10, by using N-(benzyloxycarbonyl)-L-aspartic acid β-tert-butyl ester dicyclohexylamine salt as starting material.
TLC (chloroform : acetic acid = 95 : 5) : Rf 0.31;
IR : ν 3290, 3040, 1700, 1645, 1600, 1520, 1440, 1200, 1170, 910, 755, 690 cm^{-1}.
The following desired compound was obtained by the same procedure as above.

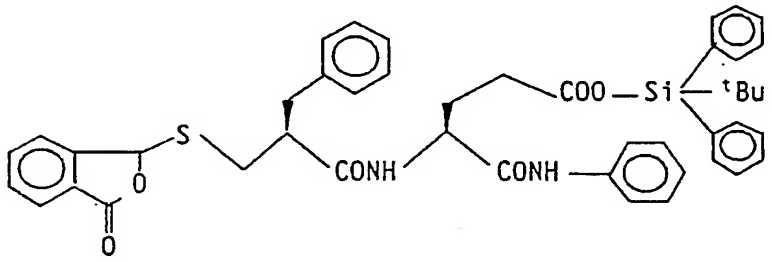
(1) N-(3-benzoylthio-2S-benzylpropionyl)-α-anilino-L-glutamic acid



TLC (chloroform : acetic acid = 95 : 5) : Rf 0.29;
IR : ν 3270, 3050, 1705, 1655, 1640, 1600, 1530, 1490, 1445, 1205, 910, 750, 685 cm^{-1}.

Reference Example 7

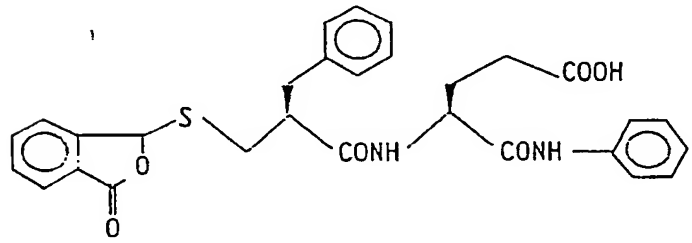
N-[3-(phthalid-3-yl)thio-2S-benzylpropionyl]-α-anilino-L-glutamic acid γ-tert-butyldiphenylsilyl ester



To a solution of the thioether compound (178 mg, prepared by the same procedure as Reference Example 5 by using the compound prepared in Example 8 as starting material) in acetone (2 ml) were added 3-phthalidyl chloride (56.1 mg) and potassium carbonate (46 mg) at room temperature, and the mixture was stirred for 2 hours at the same temperature. The reaction mixture was diluted with a mixture of ethyl acetate and ether, washed with water and a saturated aqueous solution of sodium chloride, successively, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate: n-hexane = 1 : 2) to give the title compound (153.6 mg) having the following physical data :
TLC (ethyl acetate : n-hexane = 1 : 2) : Rf 0.2.

Example 16

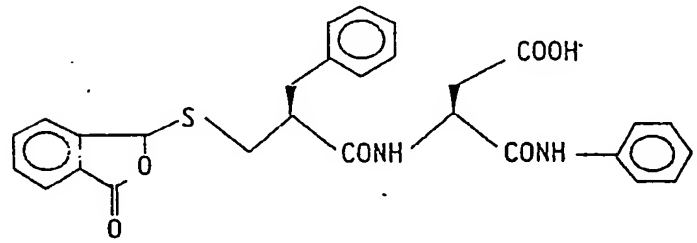
N-[3-(phthalid-3-yl)thio-2S-benzylpropionyl]-α-anilino-L-glutamic acid



The title compound having the following physical data was obtained by the same procedure as Example 13, by using the silyl ester prepared in Reference Example 7 as starting material.
TLC (chloroform : acetic acid = 95 : 5) : Rf 0.40;
IR : ν 3255, 1750, 1640, 1600, 1520, 1440 cm^{-1}.

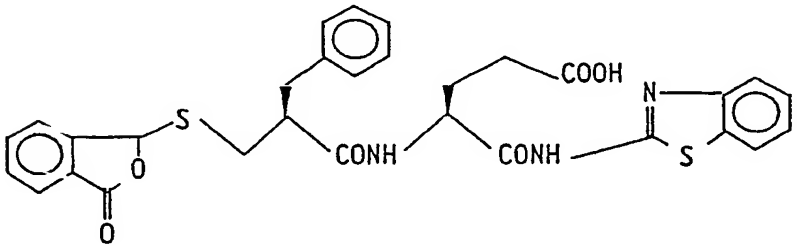
The following desired compounds were obtained by the same procedure as above.

(1) N-[3-(phthalid-3-yl)thio-2S-benzylpropionyl]-α-anilino-L-aspartic acid



TLC (chloroform : tetradhydrofuran : acetic acid = 15 : 4 : 1) : Rf 0.51;
IR : ν 3280, 3030, 1760, 1640, 1600, 1525, 1440, 1285, 1170, 940, 760, 725, 695 cm^{-1}.

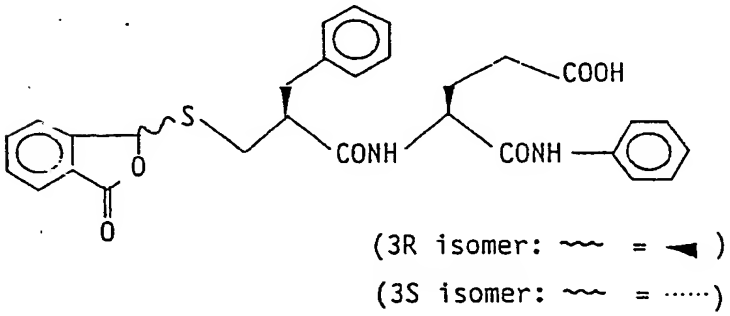
(2) N-[3-(phthalid-3-yl)thio-2S-benzylpropionyl]-L-glutamic acid α-(2-benzthiazolyl)amide



TLC (chloroform : acetic acid = 98 : 2) : Rf 0.25;
IR : ν 3300, 1760, 1640, 1600, 1540, 1440 cm^{-1}.

Example 17

N-[3-(phthalid-3R(or-3S)-yl)thio-2S-benzylproplonyl]-α-anilino-L-glutamic acid



The diastereomer (i.e. 3RS-mixture, prepared in Example 16) was recrystallized twice from ethyl acetate to give the 3R-isomer having the following physical data. Further, mother liquid obtained in the separation of R-isomer, was concentrated under reduced pressure and the residue was dissolved in ethanol and recrystallized twice from ethanol to give the 3S-isomer having the following physical data.

(1) 3R-isomer
TLC (methylene chloride : methanol = 9 : 1) : Rf 0.14;
IR : ν 3270, 3060, 1758, 1732, 1671, 1646, 1531, 1446, 1295, 1177, 960, 756, 697 cm^{-1}.

(2) 3S-isomer
Melting point: 174° ~ 176°C;
TLC (methylene chloride : methanol = 9 : 1) : Rf 0.12;
IR : ν 3286, 3060, 1774, 1709, 1677, 1639, 1533, 1445, 1290, 953, 726, 701 cm^{-1}.

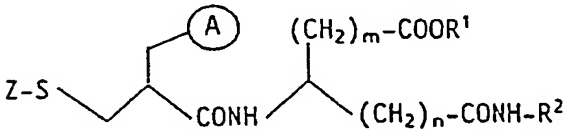
Formulation Example

The following components were admixed in conventional manner and punched out to obtain 100 tablets each containing 50 mg of active ingredient.

- N-(3-mercapto-2RS-benzylpropionyl)-α-anilino-L-glutamic acid . . . . . 5.0 g
- Cellulose calcium glycolate (disintegrating agent) . . . . . 0.2 g
- Magnesium stearate (lubricating agent) . . . . . 0.1 g
- Microcrystalline cellulose . . . . . 4.7 g

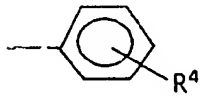
Claims

1. An amino acid derivative of the general formula:



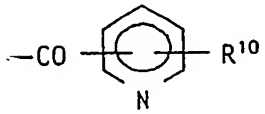
(I)

wherein
R^1 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
R^2 represents a carbocyclic or heterocyclic ring, unsubstituted or substituted by 1 to 3 substituents R^3,
R^3 represents independently;
(1) a halogen atom,
(2) a trihalomethyl group,
(3) a hydroxy group,
(4) an alkyl group of 1 to 15 carbon atoms,
(5) an alkoxy group of 1 to 4 carbon atoms,
(6) an alkylthio, alkylsulfinyl or alkylsulfonyl group, of 1 to 4 carbon atoms,
(7) a group of the formula:

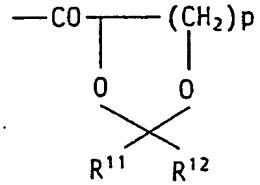


in which R^4 represents a hydrogen atom, a halogen atom, a trihalomethyl group, a hydroxy group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms
(8) a group of the formula:
$-NR^5R^6$
in which R^5 and R^6 independently represent a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(9) a group of the formula:
$-CO-R^7$
in which R^7 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^4 (in which R^4 is as hereinbefore defined),
(10) a group of the formula:
$-COOR^8$
in which R^8 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(11) a group of the formula:

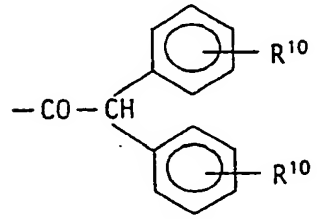
$-CONR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(12) a group of the formula:
$-SO_2NR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(13) a cyano group,
(14) a nitro group, or
(15) a group of the formula:
$-NHCO-R^7$,
in which R^7 is as hereinbefore defined,
Z represents:
(1) a hydrogen atom,
(2) a group of the formula:
$-COR^9$
in which R^9 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms, (3) a group of the formula:



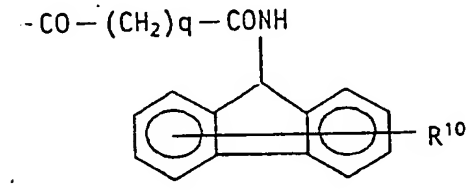
in which R^{10} is as hereinbefore defined,
(4) a group of the formula:



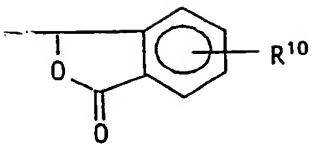
in which R^{11} and R^{12} independently represent a hydrogen atom, an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} is as hereinbefore defined, or R^{11} and R^{12} together represent an alkylene group of 4 or 5 carbon atoms and p is an integer of 1 or 2,
(5) a group of the formula:



in which the two R^{10} groups are independently as hereinbefore defined,
(6) a group of the formula:

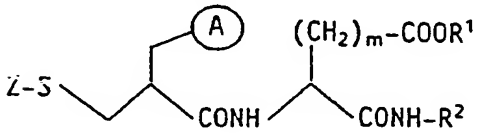


in which R^{10} is as hereinbefore defined and q is an integer of 1 to 4,
(7) a group of the formula:



in which R^{10} is as hereinbefore defined,
A represents a phenyl group or cycloalkyl group of 4 to 7 carbon atoms, each of which is substituted by R^{13}, in which R^{13} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms, and
(1) when m is zero, n is an integer of 1 to 4, and (2) when n is zero, m is an integer of 1 to 4), or a non-toxic salt thereof.

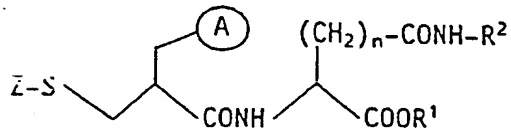
2. A compound according to claim 1 of the general formula:



(Ia)

wherein the various symbols are as defined in claim 1.

3. A compound according to claim 1 of the general formula:



(Ib)

wherein the various symbols are as defined in claim 1.

4. A compound according to claim 2 or 3, wherein Z represents a hydrogen atom.

5. A compound according to claim 4, wherein R^2 represents a carbocyclic ring unsubstituted or substituted by R^3.

6. A compound according to claim 5, wherein the carbocyclic ring is a benzene or naphthalene ring.

7. A compound according to claim 5, which is:
N-(3-mercapto-2-benzylpropionyl)-α-anilinoaspartic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methoxyanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-α-anilinoglutamic acid,
N-(3-mercapto-2-cyclohexylmethylpropionyl)-α-anilinoglutamic acid,
N-[3-mercapto-2-(4-methoxybenzyl)propionyl]-α-anilinoglutamic acid,
N-[3-mercapto-2-(4-methylbenzyl)propionyl]-α-anilinoglutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-fluoroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-chloroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(2-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(3-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methoxyanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-[4-(N,N-dimethylamino)anilino]glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-trifluoromethylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-decylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-cyanoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-carboxyanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-acetylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-nitroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-carbamoylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-sulfamoylanilino)glutamic acid or
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(1-naphthyl)amide,
a β-methyl ester of a corresponding aspartic acid derivative, a non-toxic β-carboxylate salt of a corresponding aspartic acid derivative, a γ-methyl ester of a corresponding glutamic acid derivative, or a non-toxic γ-carboxylate salt of a corresponding glutamic acid derivative.

8. A compound according to claim 4, wherein R^2 represents a heterocyclic ring unsubstituted or substituted by R^3.

9. A compound according to claim 8, wherein the heterocyclic ring represented by R^2 is monocyclic or bicyclic incorporating a benzene ring, and contains one or two nitrogen or sulfur atoms.

10. A compound according to claim 9, wherein the heterocyclic ring represented by R^2 is furan, thiophene, pyridine, pyrimidine, pyrazine, benzimidazole, benzthiazole, benzoxazole or benzodiazepine.

11. A compound according to claim 8, which is:
N-(3-mercapto-2-benzylpropionyl)aspartic acid α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide,
N-(3-mercapto-2-benzylproplonyl)glutamic acid α-(4-pyridyl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(5-chloropyridin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(pyrazin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(pyrimidin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(2-benzthiazolyl)amide,
or N-(3-mercapto-2-benzylpropionyl)glutamic acid α-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide,
a β-methyl ester of a corresponding aspartic acid derivative, a non-toxic β-carboxylate salt of a corresponding aspartic acid derivative, a γ-methyl ester of a corresponding glutamic acid derivative or a non-toxic γ-carboxylate salt of a corresponding glutamic acid derivative.

12. A compound according to claim 5, which is:
N-(3-mercapto-2-benzylpropionyl)-γ-anilinoglutamic acid,
N-(3-mercapto-2-benzylpropionyl)-β-anilinoaspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-fluoroanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-chloroanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-iodoanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-methoxyanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-[4-(N,N-dimethylamino)anilino]aspartic acid or
N-(3-mercapto-2-benzylpropionyl)-β-(4-trifluoromethylanilino)aspartic acid,
a α-methyl ester of the corresponding aspartic acid or glutamic acid derivative, or a non-toxic α-carboxylate salt of the corresponding aspartic acid or glutamic acid derivative.

13. A compound according to claim 1, which is N-(3-mercapto-2-benzylpropionyl)aspartic acid β-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide,
a α-methyl ester of the corresponding aspartic acid derivative, or a non-toxic α-carboxylate salt of the corresponding aspartic acid derivative.

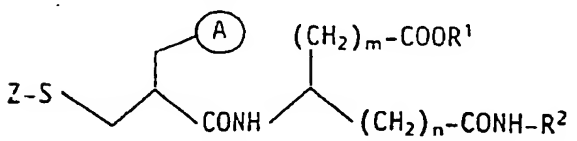
14. A compound according to claim 2 or 3, wherein Z is other than hydrogen.

15. A compound according to claim 14, which is:
N-(3-benzoylthio-2-benzylproplonyl)-α-anilino glutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)-α-anilino glutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)glutamic acid α-(2-benzthiazolyl) amide,
N-(3-diphenylacetylthio-2-benzylpropionyl)-α-anilinoglutamic acid,
N-(3-isonicotinoylthio-2-benzylpropionyl)-α-anilinoglutamic acid,
N-[3-(2,2-dimethyl-1,3-dioxolan-4-carbonyl)thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-[3-[N-(9-fluorenyl)succinamoyl]thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)-α-anilinoaspartic acid,
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]glutamic acid α-(2-benzthiazolyl)amide or
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]-α-anilinoaspartic acid,
a β-methyl ester of the corresponding aspartic acid derivative, a non-toxic β -carboxylate salt of the corresponding aspartic acid derivative, a γ-methyl ester of the corresponding glutamic acid derivative, or a non-toxic γ -carboxylate salt of the corresponding glutamic acid derivative.

16. A compound according to claim 14, which is
N-(3-acetylthio-2-benzylpropionyl)-γ-anilinoglutamic acid or
N-(3-nicotinoylthio-2-benzylpropionyl)-β-anilinoaspartic acid,
a α-methyl ester of the corresponding aspartic acid or glutamic acid derivative, or a non-toxic α-carboxylate salt of the corresponding aspartic acid or glutamic acid derivative.

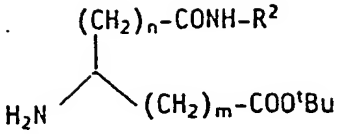
17. A compound according to any one of the preceding claims in which the amino acid derivative has the L-configuration.

18. A process for the preparation of an amino acid derivative of the general formula:



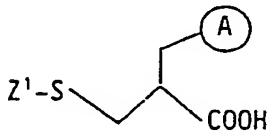
(I)

(wherein the various symbols are as defined in claim 1), which comprises:
(i) reacting to form an amide bond a compound of the general formula:



(II)

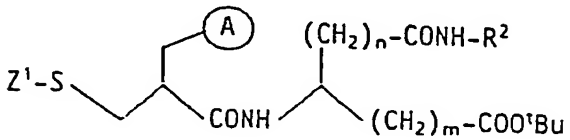
(wherein ^{t}Bu represents a tert-butyl group and the other symbols are as defined in claim 1) with a compound of the general formula:



(VI)

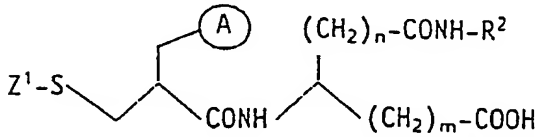
(wherein Z^1 represents a group of the formula: -COR^9 (in which R^9 is as defined in claim 1) and A is as defined in claim 1);

(ii) converting to a group -SH the group Z^1-S in a compound of the general formula:



(IA)

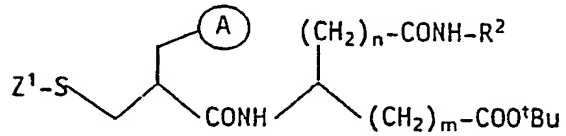
(wherein the various symbols are as defined in claim 1), or



(IC)

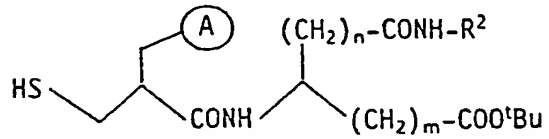
(wherein the various symbols are as defined in claim 1);

(iii) hydrolyzing to convert to a group COOH the group COO^tBu in a compound of the general formula:



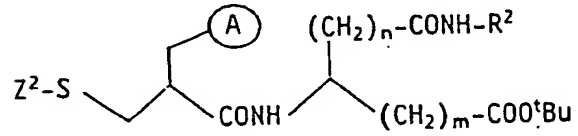
(IA)

(wherein the various symbols are as defined in claim 1),



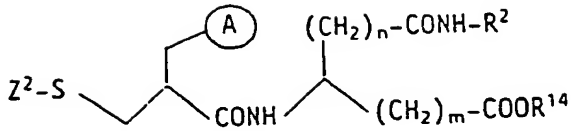
(IB)

(wherein the various symbols are as defind in claim 1), or



(IG)

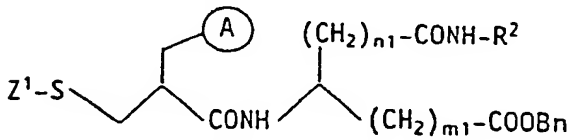
(wherein Z^2 represents the group other than a hydrogen atom in the groups represented by Z (Z is as hereinbefore defined) and the other symbols are as defined in claim 1) or to convert to a group COOH the group $COOR^{14}$ in a compound of the general formula:



(V)

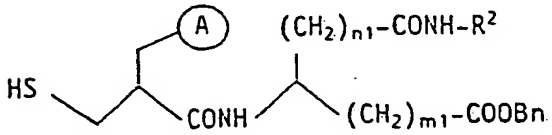
(wherein R^{14} represents a silyl group substituted by three substituents which are selected from an alkyl group of 1 to 4 carbon atoms and a phenyl group, and the other symbols are as defined in claim 1) under acidic conditions,

(iv) hydrolyzing under alkaline conditions to convert to a group SH the group Z^1S and to convert to a group COOH the groups COOBn for $COOR^{1'}$ in a compound of the general formula:



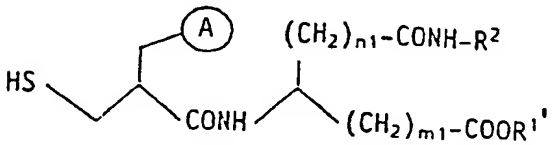
(III)

(wherein Bn represents a benzyl group and
(1) when m_1 is zero, n_1 is an integer of 1 to 4, and
(2) when n_1 is zero, m_1 is an integer of 2 to 4, and the other symbols are as defined in claim 1);



(IV)

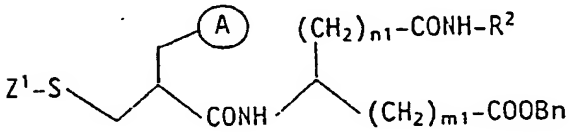
(wherein the various symbols are as defined in claim 1), or



(IF)

(wherein $R^{1'}$ is as hereinbefore defined and the other symbols are as defined in claim 1),

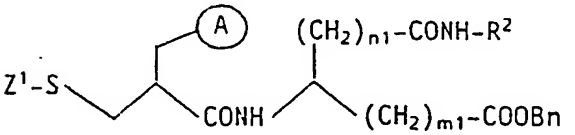
(v) converting to a group SH the group Z^1S- of a compound of the general formula:



(III)

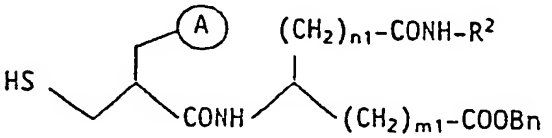
(wherein the various symbols are as defined in claim 1);

(vi) converting to a group $COOR^{1'}$ (in which $R^{1'}$ represents an alkyl group containing 1 to 4 carbon atoms) the group COOBn of a compound of the general formula:



(III)

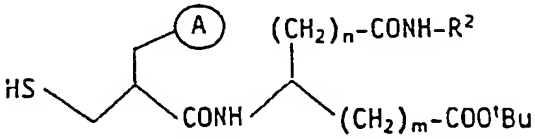
(wherein the various symbols are as defined in claim 1), or



(IV)

(wherein the various symbols are as defined in claim 1), and converting to a group -SH the group Z^1S in the compound of the general formula (III);

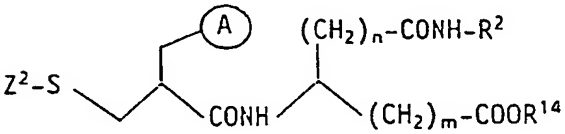
(vii) converting to a group Z^2S (in which Z^2 represents a group within the definition of Z as defined in claim 1 with the exception of hydrogen) the SH group in a compound of the general formula:



(IB)

(wherein the various symbols are as defined in claim 1);

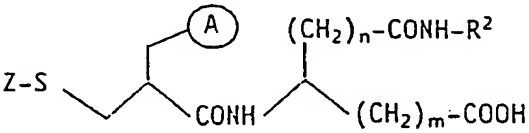
(viii) desilylating a compound of the general formula:



(V)

(wherein the various symbols are as defined in claim 1) to convert the group $COOR^{14}$ to COOH;

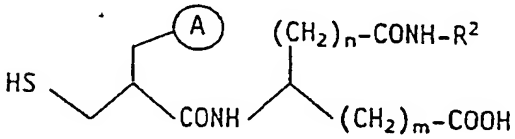
(ix) esterifying a compound of the general formula:



(IJ)

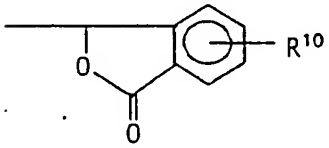
(wherein the various symbols are as defined in claim 1) to convert the group COOH to a group $COOR^{1'}$ in which $R^{1'}$ is as hereinbefore defined; or

(x) reacting a compound of the general formula:



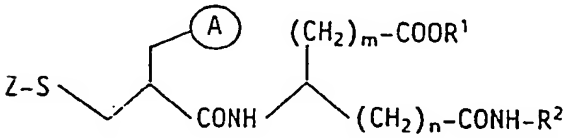
(IL)

(wherein the various symbols are as defined in claim 1) with a halide of the group of the general formula:



(wherein R^{10} is as defined in claim 1), and optionally converting an amino acid derivative of general formula (I) thus obtained into a non-toxic salt thereof.

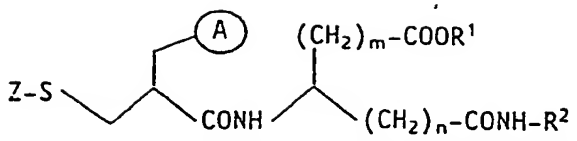
19. A pharmaceutical composition which comprises, as active ingredient, an amino acid derivative of the general formula:



(I)

(wherein the various symbols are as defined in claim 1) or a non-toxic salt thereof in association with a pharmaceutically acceptable carrier or coating.

20. An amino acid derivative of the general formula:

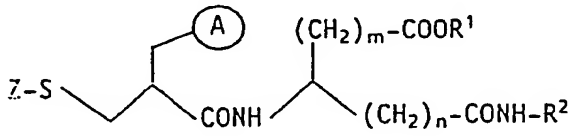


(I)

(wherein the various symbols are as defined in claim 1) or a non-toxic salt thereof, for use as an analgesic, antianxiety or anticonvulsant agent.

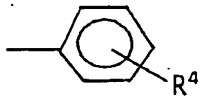
Claims for the following Contracting States : ES, GR

1. A process for the preparation of an amino acid derivative of the general formula:



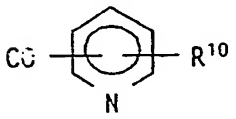
(I)

wherein
R^1 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
R^2 represents a carbocyclic or heterocyclic ring, unsubstituted or substituted by 1 to 3 substituents R^3,
R^3 represents independently;
(1) a halogen atom,
(2) a trihalomethyl group,
(3) a hydroxy group,
(4) an alkyl group of 1 to 15 carbon atoms,
(5) an alkoxy group of 1 to 4 carbon atoms,
(6) an alkylthio, alkylsulfinyl or alkylsulfonyl group, of 1 to 4 carbon atoms,
(7) a group of the formula:

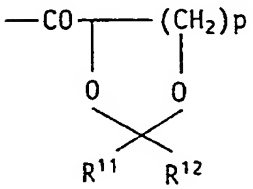


in which R^4 represents a hydrogen atom, a halogen atom, a trihalomethyl group, a hydroxy group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms
(8) a group of the formula:
$-NR^5R^6$
in which R^5 and R^6 independently represent a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(9) a group of the formula:
$-CO-R^7$
in which R^7 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^4 (in which R^4 is as hereinbefore defined),
(10) a group of the formula:
$-COOR^8$
in which R^8 represents a hydrogen atom or an alkyl group of 1 to 4 carbon atoms,
(11) a group of the formula:
$-CONR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(12) a group of the formula:
$-SO_2NR^5R^6$
in which R^5 and R^6 are as hereinbefore defined,
(13) a cyano group,
(14) a nitro group, or
(15) a group of the formula:
$-NHCO-R^7$,
in which R^7 is as hereinbefore defined,
Z represents:
(1) a hydrogen atom,
(2) a group of the formula:
$-COR^9$
in which R^9 represents an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4

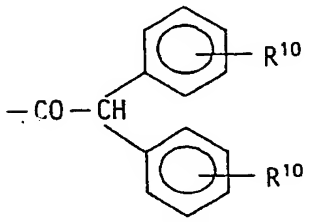
carbon atoms or an alkoxy group of 1 to 4 carbon atoms,
(3) a group of the formula:



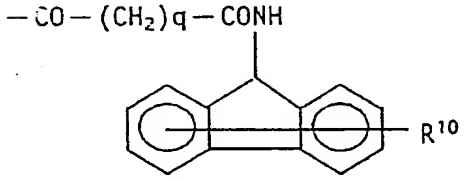
in which R^{10} is as hereinbefore defined,
(4) a group of the formula:



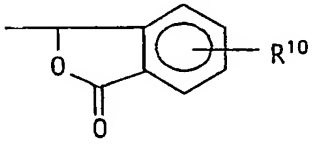
in which R^{11} and R^{12} independently represent a hydrogen atom, an alkyl group of 1 to 4 carbon atoms or a phenyl group substituted by R^{10}, in which R^{10} is as hereinbefore defined, or R^{11} and R^{12} together represent an alkylene group of 4 or 5 carbon atoms and p is an integer of 1 or 2,
(5) a group of the formula:



in which the two R^{10} groups are independently as hereinbefore defined,
(6) a group of the formula:

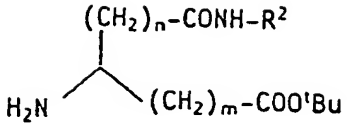


in which R^{10} is as hereinbefore defined and q is an integer of 1 to 4,
(7) a group of the formula:



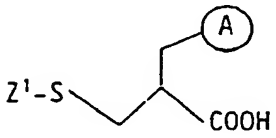
in which R^{10} is as hereinbefore defined,
A represents a phenyl group or cycloalkyl group of 4 to 7 carbon atoms, each of which is substituted by R^{13}, in which R^{13} represents a hydrogen atom, a halogen atom, a trihalomethyl group, an alkyl group of 1 to 4 carbon atoms or an alkoxy group of 1 to 4 carbon atoms, and
(1) when m is zero, n is an integer of 1 to 4, and
(2) when n is zero, m is an integer of 1 to 4), which process comprises:

(i) reacting to form an amide bond a compound of the general formula:



(II)

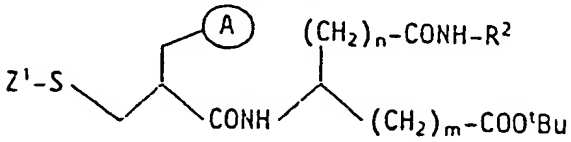
(wherein ^tBu represents a tert-butyl group and the other symbols are as defined in claim 1) with a compound of the general formula:



(VI)

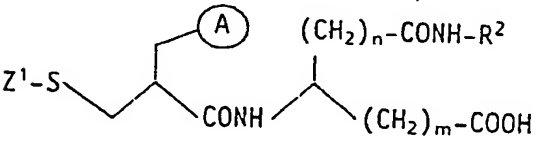
(wherein Z^1 represents a group of the formula: $-COR^9$ (in which R^9 is as defined in claim 1) and A is as defined in claim 1);

(ii) converting to a group -SH the group Z^1-S in a compound of the general formula:



(IA)

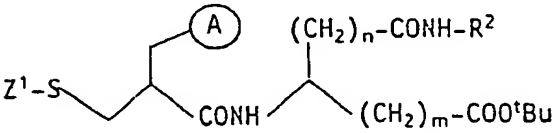
(wherein the various symbols are as defined in claim 1), or



(IC)

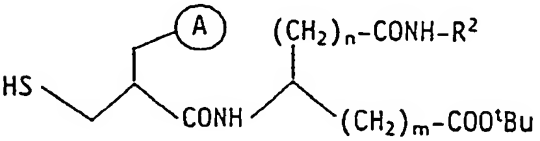
(wherein the various symbols are as defined in claim 1);

(iii) hydrolyzing to convert to a group COOH the group COO^tBu in a compound of the general formula:



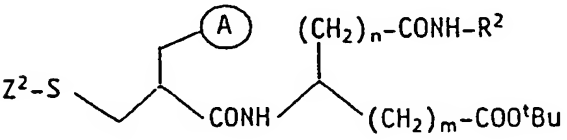
(IA)

(wherein the various symbols are as defined in claim 1),



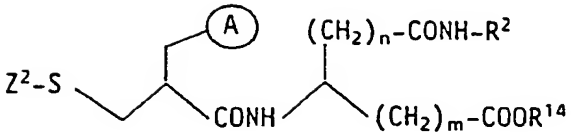
(IB)

(wherein the various symbols are as defind in claim 1), or



(IG)

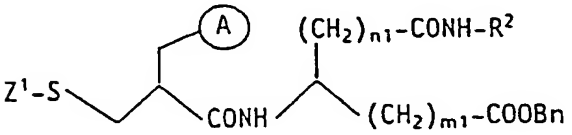
(wherein Z^2 represents the group other than a hydrogen atom in the groups represented by Z (Z is as hereinbefore defined) and the other symbols are as defined in claim 1) or to convert to a group COOH the group $COOR^{14}$ in a compound of the general formula:



(V)

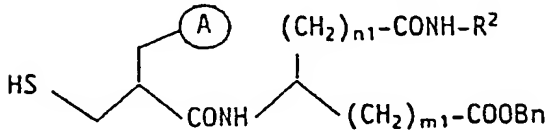
(wherein R^{14} represents a silyl group substituted by three substituents which are selected from an alkyl group of 1 to 4 carbon atoms and a phenyl group, and the other symbols are as defined in claim 1) under acidic conditions,

(iv) hydrolyzing under alkaline conditions to convert to a group SH the group Z^1S and to convert to a group COOH the groups COOBn for $COOR^{1'}$ in a compound of the general formula:



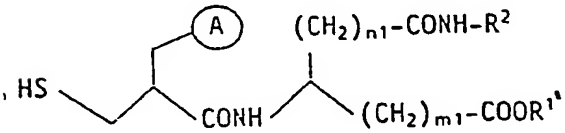
(III)

(wherein Bn represents a benzyl group and
(1) when m_1 is zero, n_1 is an integer of 1 to 4, and
(2) when n_1 is zero, m_1 is an integer of 2 to 4, and the other symbols are as defined in claim 1);



(IV)

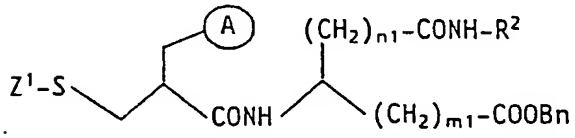
(wherein the various symbols are as defined in claim 1), or



(IF)

(wherein $R^{1'}$ is as hereinbefore defined and the other symbols are as defined in claim 1),

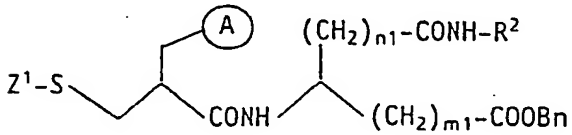
(v) converting to a group SH the group Z^1S- of a compound of the general formula:



(III)

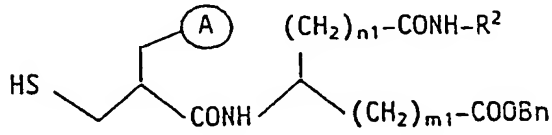
(wherein the various symbols are as defined in claim 1);

(vi) converting to a group $COOR^{1'}$ (in which $R^{1'}$ represents an alkyl group containing 1 to 4 carbon atoms) the group COOBn of a compound of the general formula:



(III)

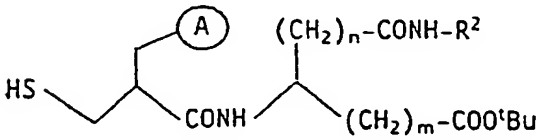
(wherein the various symbols are as defined in claim 1), or



(IV)

(wherein the various symbols are as defined in claim 1), and converting to a group -SH the group Z^1S in the compound of the general formula (III);

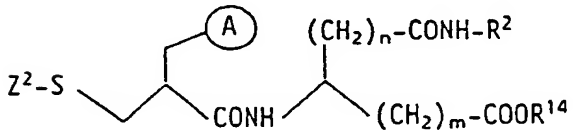
(vii) converting to a group Z^2S (in which Z^2 represents a group within the definition of Z as defined in claim 1 with the exception of hydrogen) the SH group in a compound of the general formula:



(IB)

(wherein the various symbols are as defined in claim 1);

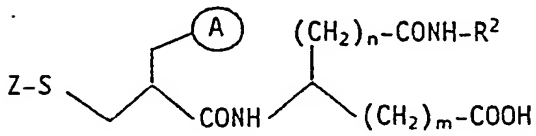
(viii) desilylating a compound of the general formula:



(V)

(wherein the various symbols are as defined in claim 1) to convert the group $COOR^{14}$ to COOH;

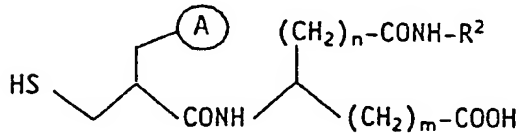
(ix) esterifying a compound of the general formula:



(IJ)

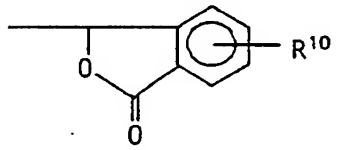
(wherein the various symbols are as defined in claim 1) to convert the group COOH to a group $COOR^{1'}$ in which $R^{1'}$ is as hereinbefore defined; or

(x) reacting a compound of the general formula:



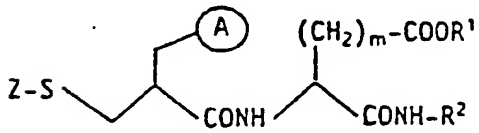
(IL)

(wherein the various symbols are as defined in claim 1) with a halide of the group of the general formula:



(wherein R^{10} is as defined in claim 1), and optionally converting an amino acid derivative of general formula (I) thus obtained into a non-toxic salt thereof.

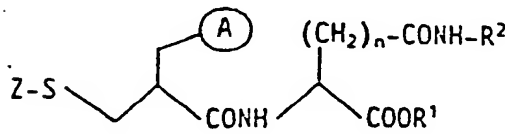
2. A process according to claim 1 for the preparation of a compound of the general formula:



(Ia)

wherein the various symbols are as defined in claim 1.

3. A process according to claim 1 for the preparation of a compound of the general formula:



(Ib)

wherein the various symbols are as defined in claim 1.

4. A process according to claim 2 or 3, wherein Z represents a hydrogen atom.

5. A process according to claim 4, wherein R^2 represents a carbocyclic ring unsubstituted or substituted by R^3.

6. A process according to claim 5, wherein the carbocyclic ring is a benzene or naphthalene ring.

7. A process according to claim 5, for the preparation of a compound which is:
N-(3-mercapto-2-benzylpropionyl)-α-anilinoaspartic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methoxyanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-α-anilinoglutamic acid,
N-(3-mercapto-2-cyclohexylmethylpropionyl)-α-anilinoglutamic acid,
N-[3-mercapto-2-(4-methoxybenzyl)propionyl]-α-anilinoglutamic acid,
N-[3-mercapto-2-(4-methylbenzyl)propionyl]-α-anilinoglutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-fluoroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-chloroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(2-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(3-iodoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methoxyanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-[4-(N,N-dimethylamino)anilino]glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-trifluoromethylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-methylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-decylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-cyanoanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-carboxyanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-acetylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-nitroanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-carbamoylanilino)glutamic acid,
N-(3-mercapto-2-benzylpropionyl)-α-(4-sulfamoylanilino)glutamic acid or
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(1-naphthyl)amide,
a β-methyl ester of a corresponding aspartic acid derivative, a non-toxic β-carboxylate salt of a corresponding aspartic acid derivative, a γ-methyl ester of a corresponding glutamic acid derivative, or a non-toxic γ-carboxylate salt of a corresponding glutamic acid derivative.

8. A process according to claim 4, wherein R^2 represents a heterocyclic ring unsubstituted or substituted by R^3.

9. A process according to claim 8, wherein the heterocyclic ring represented by R^2 is monocyclic or bicyclic incorporating a benzene ring, and contains one or two nitrogen or sulfur atoms.

10. A process according to claim 9, wherein the heterocyclic ring represented by R^2 is furan, thiophene, pyridine, pyrimidine, pyrazine, benzimidazole, benzthiazole, benzoxazole or benzodiazepine.

11. A process according to claim 8, for the preparation of a compound which is:
N-(3-mercapto-2-benzylpropionyl)aspartic acid α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(4-pyridyl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(5-chloropyridin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(pyrazin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(pyrimidin-2-yl)amide,
N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(2-benzthiazolyl)amide,
or N-(3-mercapto-2-benzylpropionyl)glutamic acid α-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide,
a β-methyl ester of a corresponding aspartic acid derivative, a non-toxic β-carboxylate salt of a corresponding aspartic acid derivative, a γ-methyl ester of a corresponding glutamic acid derivative or a non-toxic γ-carboxylate salt of a corresponding glutamic acid derivative.

12. A process according to claim 5, which is:
N-(3-mercapto-2-benzylpropionyl)-γ-anilinoglutamic acid,
N-(3-mercapto-2-benzylpropionyl)-β-anilinoaspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-fluoroanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-chloroanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-iodoanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-(4-methoxyanilino)aspartic acid,
N-(3-mercapto-2-benzylpropionyl)-β-[4-(N,N-dimethylamino)anilino]aspartic acid or
N-(3-mercapto-2-benzylpropionyl)-β-(4-trifluoromethylanilino)aspartic acid,
a α-methyl ester of the corresponding aspartic acid or glutamic acid derivative, or a non-toxic α-carboxylate salt of the corresponding aspartic acid or glutamic acid derivative.

13. A process according to claim 1, for the preparation of a compound which is
N-(3-mercapto-2-benzylpropionyl)aspartic acid β-(2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepin-7-yl)amide, a α-methyl ester of the corresponding aspartic acid derivative, or a non-toxic α-carboxylate

salt of the corresponding aspartic acid derivative.

14. A process according to claim 2 or 3, wherein Z is other than hydrogen.

15. A process according to claim 14, for the preparation of a compound which is:
N-(3-benzoylthio-2-benzylpropionyl)-α-anilino glutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)-α-anilino glutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)glutamic acid α-(2-benzthiazolyl) amide,
N-(3-diphenylacetylthio-2-benzylpropionyl)-α-anilinoglutamic acid,
N-(3-isonicotinoylthio-2-benzylpropionyl)-α-anilinoglutamic acid,
N-[3-(2,2-dimethyl-1,3-dioxolan-4-carbonyl)thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-[3-[N-(9-fluorenyl)succinamoyl]thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-(3-nicotinoylthio-2-benzylpropionyl)-α-anilinoaspartic acid,
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]-α-anilinoglutamic acid,
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]glutamic acid α-(2-benzthiazolyl)amide or
N-[3-(phthalid-3-yl)thio-2-benzylpropionyl]-α-anilinoaspartic acid,
a β-methyl ester of the corresponding aspartic acid derivative, a non-toxic β -carboxylate salt of the corresponding aspartic acid derivative, a γ-methyl ester of the corresponding glutamic acid derivative, or a non-toxic γ -carboxylate salt of the corresponding glutamic acid derivative.

16. A process according to claim 14, for the preparation of a compound which is
N-(3-acetylthio-2-benzylpropionyl)-γ-anilinoglutamic acid or
N-(3-nicotinoylthio-2-benzylpropionyl)-β-anilinoaspartic acid,
a α-methyl ester of the corresponding aspartic acid or glutamic acid derivative, or a non-toxic α-carboxylate salt of the corresponding aspartic acid or glutamic acid derivative.

17. A process according to any one of the preceding claims in which the amino acid derivative has the L-configuration.

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A3

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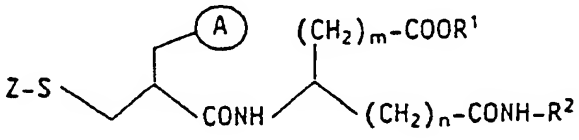
(71) Applicant: ONO PHARMACEUTICAL CO., LTD.
1-5, Doshomachi 2-chome
Chuo-ku Osaka 541(JP)

(72) Inventor: Kawamura, Masanori Ono Pharmaceutical Co. Ltd.
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka(JP)
Inventor: Arai, Yoshinobu Ono Pharmaceutical Co. Ltd.
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka(JP)
Inventor: Aishita, Hideki Ono Pharmaceutical Co. Ltd.
Minase Research Institute 1-1, Sakurai 3-chome
Shimamoto-cho Mishima-gun Osaka(JP)

(74) Representative: Bentham, Stephen et al
J.A. Kemp & Co. 14 South Square Gray's Inn
London WC1R 5EU(GB)

(54) Novel amino acid derivatives.

(57) Compounds of the formula:



(wherein R^1 is hydrogen or alkyl; R^2 is an optionally substituted carbocyclic or heterocyclic ring; Z is hydrogen or a group $-COR^3$, (R^3 is alkyl or phenyl)

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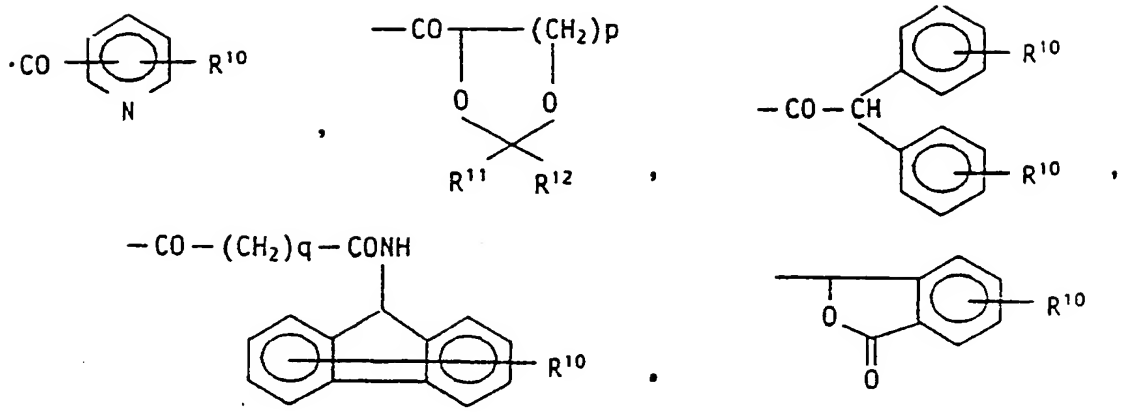

in which R^{10} is hydrogen, halogen, trihalomethyl, alkyl or alkoxy, R^{11} and R^{12} are each hydrogen, alkyl or phenyl substituted by R^{10}, or R^{11} and R^{12} together represent alkylene and p is 1 or 2; q is 1 to 4; A is phenyl or cycloalkyl optionally substituted by halogen, trihalomethyl, alkyl or alkoxy; and m is zero and n is 1 to 4 or n is zero and m is 1 to 4; and non-toxic salts thereof, have an inhibitory effect on enkephalinase and are useful as analgesic, antianxiety and anticonvulsant agents.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 4564

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	EP-A-0 136 883 (SQUIBB)		C 07 C 149/273 A 61 K 31/215 C 07 D 213/75 C 07 D 277/82 C 07 D 239/42 C 07 D 243/16 C 07 D 213/83 C 07 C 153/09 C 07 C 153/11 C 07 C 143/58 C 07 D 241/20
D,A	US-A-4 401 677 (R. GREENBERG et al.)		
A	EP-A-0 115 997 (ROUSSEL-UCLAF)		
A	GB-A-2 054 586 (USV PHARMACEUTICAL)		

TECHNICAL FIELDS SEARCHED (Int. Cl.4)
C 07 C 149/00 C 07 C 143/00 C 07 C 153/00 C 07 D 213/00 C 07 D 277/00 C 07 D 239/00 C 07 D 243/00 C 07 D 241/00 C 07 D 307/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	05-10-1990	ZAROKOSTAS K.

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